A HISTOCHEMICAL STUDY OF THE HUMAN ENDOMETRIUM AND PLACENTA IN HEALTH AND DISEASE

by

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A C K N O W L E D G M E N T S

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INTRODUCTION

Histochemistry is a new science which is gaining much interest, as it can be of great help in understanding the physiological behaviour of tissues in health and disease. It entails an integration of the hitherto separate chemical and morphological methods of scientific study, and attempts to correlate and interrelate morphology, function and chemical machinery by "chemical characterisation of morphological elements" (Dempsey and Wislocki, 1946). By histochemical means we can say whether a certain substance is present or not, and can localise its site in a given tissue. For exact quantitative determination of a substance, however, pure biochemical methods are, of course, the answer; yet histochemistry often indicates the density or diminution of a certain substance in its sites of location in a tissue, under various phases and conditions of health and disease.

In spite of the warning given by Lison in 1936 (quoted by Dempsey and Wislocki, 1946) that many reactions are valueless because they lack specificity to a certain substance, yet even in such instances significant conclusions can be derived by comparing and analysing several different lines of evidence.

In the literature, there are few records of histochemical studies on the human endometrium and placenta.

In this study, I have tried to get as much information as possible about the human endometrium and placenta, in normal and abnormal conditions, using histochemical methods. At the beginning of this work much time was spent in trying the histochemical methods known for the substances under investigation, and choosing the most convenient ones.

Seven substances have been investigated in the endometrium. They are: alkaline phosphatase, acid phosphatase, non-specific esterase, ribonucleoprotein, glycogen, mucin and lipoids. In the placenta, calcium was investigated in addition.

MATERIAL

ENDOMETRIUM

The endometrium was obtained either from curettings or from hysterectomy specimens. Material from each case was divided between five small bottles containing fixatives, immediately after operation. The fixatives are:

1. Absolute acetone, for demonstrating ensymes by the paraffin methods.
2. Zenker's fluid, for ordinary haematoxylin and eosin sections, for detection of mucin and of ribonucleoprotein.
3. Rossman's fluid, for glycogen.
4. Neutral formalin 10%, for frozen sections, to detect enzymes.
5. Neutral formalin 10%, for frozen sections to detect lipoids.

Specimens of endometrial cancer were taken from uteri removed by operation after a previous curettage had proven cancer existed. In all other conditions, the type of endometrium was diagnosed after microscopic examination of a haematoxylin and eosin section, considering the history of the case.

Normal endometrium was classified into:-

Proliferative	early	6 cases
	late	5 cases
Secretory	early	6 cases
	mid	15 cases
	late	6 cases

Abnormal endometrium comprised the following conditions:-

Anovulatory endometrium with primary sterility 6 cases.

Nonspecific chronic infection 4 cases
2 proliferative
2 secretory

Menopausal endometrium 3 cases

Cystic glandular hyperplasia 12 cases

Endometrial cancer 10 cases

Endometrial curettings were obtained from Ward 35, Royal Infirmary, Edinburgh. Hysterectomy specimens were obtained from the three gynaecological wards and the Chalmer's Annexe of the Royal Infirmary, and from the Western General Hospital, Edinburgh.

PLACENTA

Placentae from normal and abnormal pregnancies were obtained within 10-20 minutes after delivery.

Six blocks of tissue were taken from each placenta: three from the maternal surface to include decidual tissue, and three from the depth of the placenta nearer to the foetal aspect. Blcoks were t aken from different areas of apparently healthy tissue. Each block was further cut to five slices 2 x 5 x 10 mm. each approximately, to be distributed among the five bottles of fixatives. Sometimes bigger blocks were taken from the rest of the placenta, to get a better idea about the general histology or the general distribution of fat. The number of normal placentae was:-

First trimester	8
Second trimester	4
Third trimester	6
At term (39-41 weeks)	15

The number of placentae from abnormal pregnancies was:-

Post-mature (42 or more weeks)	8
Pre-eclampsia and eclampsia	18
Diabetes mellitus	10
Essential hypertension	6
Chronic nephritis	2
Hydatidiform mole	2

The clinical diagnosis was made by the staff of the Simpson Memorial Maternity Pavilion.

Cases of toxaemia were chosen from pregnancies that showed signs of toxaemia for some time in the second half of pregnancy. The signs and symptoms were either moderate or of severe degree. Cases of diabetes mellitus and essential hypertension were not complicated by pre-eclamptic toxaemia.

METHODS OF INVESTIGATION

ALKALINE PHOSPHATASE

Two methods were used for the investigation of alkaline phosphatase. They are:-

I. Calcium Cobalt Method (Gomori, 1946) with some modifications as used in the Royal Hospital for Sick Children, Edinburgh.

II. Coupling Azo - Dye Method as recommended by Pearse, 1953, with some modifications.

I. Calcium Cobalt Method for Alkaline Phosphatase

Steps:

1. Fix thin bits of tissue (2 mm. thick) in absolute acetone at 4°C, for 24 hours.
2. Dehydrate in two changes of absolute ethyl alcohol, one hour each.
3. Clear in two changes of benzene, 45 minutes each.
4. Impermeate with paraffin wax in vacuum, at pressure reduced to 400 mm. Hg., at 48°C, on two successive occasions (total time 45 minutes).
5. Embed in paraffin wax of melting point 56°C.
6. Store in refrigerator if you have to wait.

7. Blocks are trimmed and sections are cut about 6 microns thick, and mounted on slides, using warm water (about 37°C).
8. Slides are put in incubator at 37°C for two hours. They can be stored in the refrigerator if need be for one or two days.
9. Deparaffinise with benzene, then wash with absolute alcohol.
10. Treat with 1% celloidin in equal parts of absolute alcohol and ether for 2-3 minutes.
11. Change to 70% alcohol for 5 minutes, then to water.
12. Incubate the test slides with phosphate buffer pH 9.4 for 2.5 hours at 37°C and put the control slides in 0.1% calcium nitrate solution at 37°C for the same time as the slides are in the phosphate buffer, then carry out the following steps on both.
13. Rinse in water.
14. Place in 2% cobalt nitrate solution for 5 minutes.
15. Wash in distilled water for 10 minutes.
16. Place in 2% solution of freshly prepared ammonium sulphide for 2 minutes.
17. Wash in tap water.

18. Counterstain with 1% aqueous solution of Acridine Red, or 1% aqueous eosin for 5 minutes.

19. Dehydrate, clear and mount.

Stock Buffered Phosphate Substrate for Alkaline Phosphatase

50 cc of 2% sod. B. glycerophosphate
50 cc 2% sod. diethyl barbiturate
100 cc distilled water
10 cc 2% calcium chloride
5 cc 2% magnesium sulphate

Add the ingredients in the given order. This mixture gives an ultimate pH 9. With the addition of a crystal of thymol to prevent the growth of mould it can be kept in the refrigerator for months.

II. Modified Coupling Azo Dye Method for Alkaline Phosphatase

The following is the way in which the method was carried out: the diazonium salt used is 4-benzoyl amino-2:5-dimethoxy aniline (Light and Co.).

Steps:

1. Fix thin slices of tissue in 10% neutral formalin at 4°C for 24 hours.
2. Wash in water for 30 minutes.
3. Embed in gelatine for one hour at 37°C.

Embedding gelatine:

Gelatine	15 g
Glycerine	15 ml
Distilled water	70 ml

A crystal of thymol is added.

4. Cool and harden in 40% formalin at room temperature for one hour.

5. Leave the gelatine block in the refrigerator for 24-48 hours to get firm enough for cutting by the freezing microtome.

6. Cut frozen sections 15 microns thick.

7. Prepare a veronal acetate buffer of pH 9.16 as follows:

Take 10 ml. of Michaelis stock veronal buffer, which is prepared as follows (Pearse):

9.714 g. sod. acetate 3 H_2O

14.714 g. sod. barbiturate

in carbon dioxide free distilled water to 500 ml.

To these 10 ml. add

4 ml. 8.5% NaCl solution

0.5 ml. N/10 HCl,

then add distilled water (carbon dioxide free) up to 50 ml.

8. Weigh 20 mgm. sod. -naphthyl phosphate, and 40 mgm. of diazonium salt.
9. Add the 20 mgm. of the first salt to 40 ml. of the freshly prepared veronal acetate buffer, shake well, then add the 40 mgm. of the diazonium salt. Shake and filter.
10. Transfer the sections into the freshly prepared and filtered solution of step 9; leave at room temperature for 30 minutes.
11. Transfer the sections into distilled water and leave for some time to allow air bubbles to get out of the sections.
12. Either mount directly on a clean slide, or counterstain the nuclei with Grenacher's alum carmine for 10 minutes; wash with water and mount.
13. Leave the slides till excess water has drained but keep the sections wet.
14. Cover with sover slide, using glycerine jelly.

Preparation of glycerine jelly:

Dissolve 15 gm. best quality white gelatine in 100 ml. distilled water, with moderate heating.

Add 100 gm. glycerine and warm for 5 minutes on a water bath; filter while hot through glass wool.

To each 100 ml. of the mixture add one drop of phenol (phenol liquefactum B.P.U.S.P.).

Put in the incubator at 37°C till air gets out of the solution, then cover the bottle.

Preparation of Grenacher's Alum Carmine:

Carmine	4 gm.
Ammonium alum	8 gm.
Distilled water	20 ml.

Boil for one hour.

Add water to make up to the original volume.

When cool, filter and add a crystal of thymol.

ACID PHOSPHATASE

I. Lead Phosphate Method of Gomori, 1950:-
(as described by Lillie, 1952, with some modifications)

Sections from the paraffin blocks prepared for the calcium cobalt method for alkaline phosphatase are used to demonstrate acid phosphatase by the lead phosphate method.

Slides were carried through the same steps as those for alkaline phosphatase, Method I, till the stage of putting in the specific buffered substrate.

For acid phosphatase by the lead phosphate method two buffers were used, one at pH 4.8 and another at pH 5.2,

as the optimum pH for acid phosphatase differs according to the amount of the enzyme present in the tissue tested, which we do not know beforehand.

Buffers were prepared according to Walpole's table (Lillie, 1952).

How to get an acetate buffer of final pH 4.8:

We first make the buffer for pH 5.11 by adding 5 ml. M/5 acetic acid to 15 ml. M/5 sodium acetate. When the various chemicals were added to the buffer, the pH was slightly lowered and came down to 5.

If we use the buffered substrate solution the same day it is prepared we get a precipitation on the slides which spoils the reaction, so we have to leave the buffered substrate solution to stand overnight, and then filter and use for incubating the slides tested. The final pH of the buffered substrate then is again lowered to 4.8.

How to get a buffered substrate of final pH 5.2:

First take the formula for pH 5.574 as such:

2 ml. M/5 acetic acid + 18 ml. M/5 sodium acetate.

After addition of the various chemicals the pH falls to 5.3.

When this is left overnight and then filtered, the ultimate pH is 5.2.

The buffered substrate had to be prepared freshly each time the day before use; or else the substrate becomes inactive if left for some time, probably due to precipitation of glycerophosphate.

How to prepare the actual substrate solution:

The method as given by Cowdry is:

Acetate buffer	12 ml.
Lead nitrate (0.1 M)	10 ml.
Distilled water	74 ml.
Sodium B glycerophosphate (3.2%)	4 ml.

added in given order.

Shake well. Heat to about 60°C for about 10 minutes; then filter. Leave the filtrate overnight, then filter again, and use for incubation.

II. Coupling Azo Method for Acid Phosphatase:-

The azo method used for acid phosphatase is based on Burton's (1954) modification of Seligman's method.

Steps:

Sections from the gelatine embedded tissue for the azo method of alkaline phosphatase were used for acid phosphatase demonstration by the azo method named above.

Prepare the fresh substrate medium as follows:

Add 8 mgm. of sodium naphthyl phosphate to 40 ml. of acetate buffer pH 5.7 (Lillie, 1952; p.263); this is made by adding

1.5 ml. M/5 acetic acid to

18.5 ml. M/5 sodium acetate, then adding

20 ml. distilled water (carbon dioxide free).

Shake well, then add 40 mgm. of the diazonium salt, 4-benzoyl amino 2:5-dimethoxyaniline (the salt used by Burton was tetrazolised di-O-anisidine, but it has the disadvantage of giving a brown colour to the whole background)*.

Shake again and filter.

Put the frozen sections to be examined in the substrate solution in a Petri dish, cover and incubate at 37°C for one hour.

Wash in distilled water; transfer into another change of distilled water; cover and leave at room temperature overnight to allow the air bubbles to come out of the sections.

Mount on clean slides as such, or counterstain the nuclei with Grenacher's alum carmine for 10 minutes, wash

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*(Sodium -naphthyl phosphate and the diazonium salt were obtained from Light and Co.).

in water and mount in glycerine jelly.

NONSPECIFIC ESTERASE

-Naphthyl Acetate Azo Coupling Method:- (done on frozen and paraffin sections (Pearse, 1953)).

The following points are to be noted:

1. The reaction given by this method represents the activity of lipase, cholinesterase and acetylcholinesterase.
2. The frozen and paraffin sections for nonspecific esterase were taken from the gelatine and paraffin blocks used for alkaline phosphatase and acid phosphatase.
3. The diazonium salt used in this study is 4-benzoyl amino-2:5-dimethoxyaniline (Light and Co.).

The substrate solution was prepared as follows:

1. Add 50 mgm. of the diazonium salt to 50 ml. 0.1 M phosphate buffer pH 7.4 (which is made by adding 10 ml. K H_2phosphate to 40 ml. Na_2H phosphate).
2. Shake thoroughly till most of the initial cloudiness disappears.

3. Dissolve 50 mgm. -naphthyl acetate in 0.5 ml. acetone.
4. Alternately add a few drops of -naphthyl acetate in acetone to a few ml. of the buffered diazonium salt solution.
5. Filter. The filtrate will be the buffered substrate enough for one group of sections, either the paraffin or the frozen ones, and has to be used immediately.

The frozen sections are incubated in the substrate solution for 15 minutes in a covered Petri dish at room temperature, while the paraffin sections after being deparaffinised, are incubated in the substrate solution in a Coplin jar for one hour, also at room temperature.

After incubation, sections are washed with water, then mounted using glycerine jelly, either with or without counterstaining with Grenacher's alum carmine.

The reaction is represented by a black deposit.

RIBONUCLEOPROTEIN

Eosin Methylene Blue Method using Ribonuclease Controls:- (Carleton, 1938; Pearse, 1953).

Procedure:

1. Fix in Zenker's fluid, then wash in water.

2. Dehydrate, clear, impermeate and embed in paraffin.

3. Cut sections 5-6 microns thin, mount them on clean glass slides, incubate at 37°C for at least three hours, to ensure adherence of the sections to the slides.

4. Deparaffinise, then carry the slides through descending grades of alcohol to water.

5. Control slides are incubated for one hour at 37°C in a solution of crystalline ribonuclease in glass distilled water (0.5 to 1 mgm./ml.), then washed in running water.
(Crystalline ribonuclease was obtained from Light and Co., and kept at 4°C).

6. The control and test slides are stained with eosin and methylene blue, as described by Carleton.

The basophilic material removed by ribonuclease is taken to be ribonucleoprotein.

Method of staining with Eosin and Methylene Blue:
(described by Carleton, 1938).

1. Flood the surface of the slides with 5% aqueous solution of eosin. Hold over a Bunsen burner until water vapour just begins to come off. Leave for 1-3 minutes.

2. Rinse very rapidly in tap water. By now the sections should have been deeply stained.

3. Flood the slides with Borrel's methylene blue, diluted in distilled water one volume to five. Warm over Bunsen burner, and lay aside as done with eosin staining.

4. Rinse in tap water. The sections should be blue black at this stage.

5. Differentiate in:

absolute alcohol	50 ml.
acetone saturated with colophonium resin	50 ml.

Both eosin and methylene blue destain very rapidly, but the former resists the alcohol more than the basic dye. A stage is reached when the cell nuclei are of a bright transparent blue colour, and the cell cytoplasm, muscle and red blood corpuscles are bright pink. This is the point of optimum differentiation.

6. Complete dehydration by dipping into absolute acetone or absolute ethyl alcohol.

7. Clear in xylol.

8. Mount in canada balsam.

Result:

Cell nuclei are blue; cytoplasm, collagen connective tissue fibres and muscle are pink; red blood corpuscles are bright pink, as also are the oxyphil granules of the eosinophil leucocytes. In the course of time methylene blue fades somewhat.

Notes:

1. It is important that the sections should be grossly overstained in eosin, then in methylene blue.
2. Speed in dehydration and in control of the degree of differentiation is essential. The colophonium affords a sharper differentiation.
3. If the sections are understained or over-differentiated (which amounts to the same thing), take them down through descending alcohols (which will complete extraction of the stain) to water, then start again.
4. A good sample of water-soluble eosin is essential.

Preparation of Borrel's Methylene Blue:

A. Make a 1% solution of methylene blue (chemically pure) in distilled water by heating over water bath.

B. Dissolve 0.5 gm. of silver nitrate in 100 ml. distilled water in a perfectly clean flask of about 500 cc. capacity. Gradually add to this a 3% solution of sodium or potassium hydroxide, shaking the flask after each addition of the alkali. A brown precipitate of silver oxide is formed. Stop adding the alkali as soon as no more precipitate comes down. Allow the latter to settle, decant the supernatent fluid, and wash the precipitate by washing it with distilled water.

Repeat the process until the distilled water of the washing is no longer alkaline to litmus paper. Pour off the distilled water.

Add solution "A" to the silver oxide. Boil gently for 5 minutes and allow to cool. The solution should be dark violet when cold. If still blue, boil it again to oxidise the methylene blue still further. If it be distinctly reddish, add a little 1% methylene blue until it becomes violet. Usually the solution is of the correct colour if prepared as described above. Filter and store in a stoppered bottle. The solution keeps well.

For use, dilute by adding 5 volumes of distilled water to each volume of the solution.

NB: The flasks must be perfectly clean and washed with distilled water. Pure reagents are essential. Suitable varieties of methylene blue are:

methylene blue medicinal, or methylene blue, rectified according to Ehrlich and Grüber, now Hollborn,

GLYCOGEN

Glycogen was investigated by two methods:-

I. Best's Carmine stain (Pearse, 1953).

II. Chromic acid - Schiff (Lillie, 1952).

Rossman's fluid was used as fixative, and paraffin sections prepared for either method from the same block.

I. Best's Carmine Stain:-

Preparation of solutions:

Carmine Stock Solution:

Add 2 gm. carmine, 1 gm. potassium carbonate and 5 gm. KCl , to 60 ml. distilled water. Boil gently for 5 minutes, cool and filter. Add to the filtrate 20 ml. of ammonia (specific gravity 0.88).

This solution lasts three months at -4°C. (I have found it to keep only for one month).

Carmine Staining Solution:

Dilute 15 ml. of the stock solution with 12.5 ml. of ammonia (specific gravity 0.88), and 12.5 ml. of methyl alcohol. This solution lasts for 2-3 weeks.

Best's Differentiator:

Absolute ethyl alcohol	8 ml.
Absolute methyl alcohol	4 ml.
Aqua distillata	10 ml.

Method of Staining:

1. Bring sections to absolute alcohol.
2. Place sections in 1% celloidin in absolute alcohol and ether (equal parts) for 2 minutes.
3. Dry in air.
4. Pass through alcohols to water.
5. Stain in Eherlich's haemalum for 5 minutes.
6. Rinse and differentiate rapidly in 1% acid alcohol.
7. Rinse in water.
8. Stain in Best's carmine solution for 15-30 minutes.
9. Differentiate in Best's differentiator, without previous rinsing for 5-60 seconds.
10. Wash in 80% alcohol.

11. Dehydrate in absolute alcohol, clear in xylene and mount in D.P.X. (Depex, Gurr's Mounting Medium).

Result:

Nuclei dark blue, glycogen red.

NB: Control slides after deparaffinisation are brought down to water, incubated with saliva at 37°C for 30 minutes on three occasions, then washed in water, dehydrated and carried through the same steps as the test slides. Any red colour in the test slide is not considered glycogen.

II. Chromic Acid Schiff Method:-

The method used in this study is the one described by Lillie (1952).

The Schifff reagent is prepared as follows:

Dissolve 1 gm. basic fuchsin in 100 ml. hot (90°-95°C) distilled water. Filter at 50°-60°C as it cools. Cool and add 2 gm. sodium bisulphite ($NaHSO_3$) and 20 ml. N/1 hydrochloric acid. Stopper tightly and store in dark overnight at room temperature. Add 300 mg. finely powdered charcoal, shake 1 minute, and then filter. The solution should now

be clear light yellow. Store at 5°C. When kept at this temperature it remains active for some months. When any pink tint appears, it should be discarded.

Method of Staining:-

1. Bring the sections up to absolute alcohol.
2. The digestion control slides are passed through 95% and 80% alcohol to water. Wash out the picric acid used in the fixative, digest for half an hour at 37°C. Dehydrate by passing through 95% and 100% alcohol.
3. The test and the control slides are put in 1% celloidin in ether and 100% alcohol (50:50) for 5 minutes.
4. Stand slides on edge vertically and let drain 1 minute.
5. Harden 5 minutes or longer in 80% alcohol.
6. Rinse in water and oxidise for one hour in a coplin jar with 40 ml. 5% fresh chromic anhydride solution (Cr O 3).
7. Wash for 5 minutes in running water.
8. Treat with 40 ml. Schiff's reagent in a covered coplin jar for 15 minutes, agitating the fluid every 3-5 minutes. (The Schiff reagent may

be re-used several times in the same day, but should not be returned to the stock bottle).

9. Pass through 3 changes, 90 seconds each, of M/20 sodium bisulphite ($NaHSO_3$). This is made extempore by diluting 6 ml. of a stock molar (10.4%) solution with 114 ml. tap water, which gives 3 portions of 40 ml. each for 3 coplin jars. These solutions may be used several times on the same day.
10. Wash 10 minutes in running water.
11. Stain 2-3 minutes in a very dilute light green solution (dilute the 1% solution 25 times).
12. Dehydrate with two changes each of 95% and 100% alcohol. If it is desired to remove the celloidin film use 3 changes of acetone.
13. Clear with a mixture of equal volumes of 100% alcohol or acetone and xylene followed by two or more changes of xylene. Mount in xylene clarite.

Results:

Bauer positive material red purple, the background light green. Glycogen is usually distinct from other Bauer positive materials by being granular and brighter in colour.

MUCIN

Several Considerations:

The mucin of the histologist is an intercellular secretory product formed especially in various epithelia.

Biochemically there are many mucins of slightly different chemical composition. The term 'mucin', though convenient, does not refer to a single chemical entity such as adrenalin. Histochemically, mucin has the following characteristics.

(a) Great affinity for the basic dyes in general; this is due to the acid reaction of mucin.

(b) Metachromatic staining with certain basic aniline dyes.

(c) Solubility in weak alkalis.

(d) Precipitation by acetic acid.

(a) and (c) are the basis of Southgate's Mucicarmine stain, long considered specific for mucin.

The Southgate Mucicarmine Stain (Carleton, 1938).

Preparation of the Staining Solution:

Place 1 gm. of powdered carmine and 1 gm. of dry aluminium hydroxide in a 500 ml. flask. (N.B. Cheap, commercial carmine seems to give the best results). Add 100 ml. of 50% alcohol, then 0.5 gm. of anhydrous powdered aluminium chloride whilst shaking. Place on

boiling water-bath, boil for precisely 2½ minutes.

Cool under the tap and filter when cold.

Method of Staining:

Paraffin sections of the Zenker fixed tissue are used.

1. Bring paraffin sections down to distilled water.
2. Stain the nuclei with haematoxylin.
3. Wash in water.
4. Stain for thirty to forty-five minutes.
5. Rinse in distilled water.
6. Dehydrate, clear and mount.

Result:

Mucin red, nuclei blue.

N.B.: For precise but less energetic staining, dilute the stain with distilled water and prolong the time of staining.

CALCIUM

The Von Kossa Method for Calcium Deposits (Pearse, 1953)

This method really shows carbonates and phosphates. It has always been used to show calcium in deposits, as

these deposits are usually formed of calcium phosphate or calcium carbonate.

Paraffin sections of neutral formalin fixed tissue are used for this method.

Procedure

1. Bring sections to distilled water and rinse thoroughly.
2. Immerse in 0.5% to 1% aqueous $AgNO_3$ for 10-15 minutes (leave in sunlight or ultraviolet light).
3. Rinse in distilled water.
4. Immerse in 5% aqueous sodium thiosulphate for 30 seconds.
5. Counterstain the nuclei with 1% neutral red for 30 seconds.
6. Wash in tap water.
7. Dehydrate in alcohol, clear in xylene and mount in Canada balsam or D.P.X. (the latter causes slow fading).

Result:

Phosphates and carbonates appear black, while nuclei take a red colour.

LIPOIDS

Fixation for fat was done in neutral formalin at room temperature and after washing the tissue was embedded in gelatine. The gelatine blocks were kept in 1% formalin at room temperature till the time of cutting. Frozen sections were cut of the desired thickness and washed in water before carrying out the following fat tests:

I. Sudan IV in Triethyl Phosphate (stain for neutral fat)

This stain was recommended by Mr. McKenzie of the Obstetric Department in Edinburgh after his personal communication with the people at Animal Diseases Research Station, Edinburgh. It was found to show minute droplets of fat and to give a nice red colour which makes a good contrast with the dilute haematoxylin counterstain. The only drawback of this stain, as I noticed, is that it forms a black precipitate if the slides are kept for some time.

Preparation of the Stain:

A. Make a saturated solution of Sudan IV in pure triethyl phosphate (approximately 1.5%).

B. Allow to stand overnight at room temperature.

C. Dilute to 60% with distilled water one hour before use then filter.

Procedure:

1. Cut frozen sections 15 u thick of neutral formalin-fixed, gelatin-embedded tissue, and wash in water for about 10 minutes.
2. Rinse in 60% triethyl phosphate.
3. Stain for half an hour in Sudan IV in triethyl phosphate (60%) at 37°C.
4. Rinse in 30% triethyl phosphate.
5. Wash in water.
6. Counterstain in Harris' haematoxylin solution diluted 1 in 4 volumes of water for 5 minutes.
7. Wash in water.
8. Rinse in 1% acetic acid.
9. Wash in water till the haematoxylin colour is blue.
10. Mount in glycerine jelly.

II. Schultz Test

Schultz test is an adaptation to histology of the Liebermann-Burchardt reagent. The test was at one time considered specific for cholesterol and cholesterol esters after Romeis (1928). Then the reaction was found to indicate only unsaturation in sterol molecules (Bierry and Gouzan, 1936; Fieser, 1937; and Sobotka, 1938). Pearse mentioned that Boscott and Mandle (1949) have

applied the reaction to pure samples of dihydro-iso-androsterone, progesterone, and deoxycorticosterone acetate without obtaining the characteristic blue-green colour.

In practice, however, the various histochemical modifications of the Liebermann-Burchardt reaction are used to distinguish cholesterol and its esters. The possibility that a naturally occurring sterol might give the reaction is still there.

Schultz test was performed in this study on the endometrium and placenta. When this test was positive, the digitonin reaction was done to distinguish between free cholesterol and otherwise of the related compounds. When free cholesterol was excluded, the reaction was interpreted as due to a cholesterol ester or a related steroid. Bennet in 1940 in a histochemical study of the adrenal cortical steroids, debated the probability of cholesterol being a precursor of sterones and supported the view that there is a relation somehow.

Technique of Schultz Test as modified by Mallory (Lillie, 1952)

1. Cut thin (10-15.4) frozen sections of neutral formalin fixed tissue and wash them thoroughly in water.

2. Mordant sections in a closely stoppered bottle for 3 days at 37°C in 2.5% iron alum solution to oxidise the tested substance, (cholesterol, its ester or a related steroid).

3. Rinse in distilled water, float on to slides and blot dry.

4. Treat with a few drops of acetic sulphuric mixture made as follows:
   Place 2 to 5 ml. glacial acetic acid in a small test tube and immerse in ice water. Then add gradually the same volume of concentrated sulphuric acid while the tube is still in the ice water.

5. Cover with a cover glass and examine at once.

Result:

A blue-green colour appears in a few seconds, becoming stronger in the first few minutes and often turning to brown in half an hour. (Photographs were taken immediately as the colour is not permanent).

Positive control sections of a previously tested adrenal cortex were used. At least 3 sections of the material under test were treated with the acetic sulphuric mixture and examined before considering the test negative.

III. Windau's Digitonin Reaction (Lillie, 1952)

This is a reaction for free steroids. Slides and coverslips should be absolutely clean, since cholesterol is present in sweat, and hence finger prints give positive reactions.

The technique as recommended by Cowdry is as follows:

1. Fix in formalin, cut frozen sections.
2. Immerse sections in a 0.5% solution of digitonin in 50% alcohol in a small covered dish for several hours.
3. Rinse in 50% alcohol.
4. Counterstain part of the sections only by the usual haematoxylin Sudan IV method.
5. Mount all sections as usual in glycerol gelatine.

Result:

The uncounterstained sections under polarised light show needles or rosettes of complex cholesteryl digitonids, if free cholesterol is present.

In the counterstained preparations, the cholesterol compound remains doubly refractile and does not stain, while the cholesteryl ester compound colours with the oil soluble dye and loses its birefringence.

IV. Examination of Fat with Polarised Light (Dempsey and Wislocki, 1944; Lillie, 1952)

Birefringence alone does not give definitive conclusions regarding the nature of lipoidal substances unless combined with other methods (e.g. Sudan and Schultz test).

The double refraction is more brilliant in the fresh teased preparations but fixed material gives better localisation. In this study neutral formalin-fixed frozen sections were usually used for examination under polarised light; sometimes fresh teased placental tissue was used.

According to authorities in this field, including Lillie, neutral fats ordinarily remain dark, while any fatty substance in a solid cyrstalline form may be luminous under polarised light. Substances forming Lehmann's "Liquid crystals", such as cholesteryl esters, phosphatides, and cerebrosides, may exhibit the black cross of polarisation with luminous quadrants between the arms of the cross filling out a circle.

This phenomenon is suppressed if the temperature is above that at which the liquid crystals in question can exist and the globules remain dark.

It follows, therefore, that cholesterol esters giving a positive Schultz test may not be luminous with polarised

light. When the temperature is optimum, however, they give the characteristic "Maltese Cross" polarisation.

Free cholesterol is seen as long rhomboidal crystals which glow under polarised light and are extinguished and light up alternately once in each 90° of rotation of the stage.

Other birefringence as that of connective tissue fibres and fibrillar brush border is extinguished by rotation of the stage because they are arranged parallel to each other.

V. Phenyl-hydrazine Test

This test was done in a collection of normal and pathological placentae. It is a test for ketones, which was used by some authors to help in the localisation of ketosteroids, with other fat tests carried out as well. For the description and discussion of this test, refer to Bennet (1940).

PART I

THE HUMAN ENDOMETRIUM

Observations and Discussion

OBSERVATIONS

Observations on the human endometrium will be dealt with under the following headings.

A. Normal Endometrium.

I. Early proliferative endometrium

II. Late proliferative endometrium

III. Early secretory endometrium

IV. Mid-secretory endometrium

V. Late secretory endometrium

B. Pathological Endometrium

I. Anovulatory endometrium in sterility

II. Menopausal endometrium

III. Nonspecific inflammations of the endometrium

IV. Cystic glandular hyperplasia of the endometrium

V. Endometrial carcinoma.

A. NORMAL ENDOMETRIUM

I. Early Proliferative Endometrium

The number of cases examined was six.

HISTOLOGY: (from the haematoxylin and eosin sections).

The glandular epithelium showed early signs of activity in the form of nuclear mitosis. The nuclei

in most of the glands were still present in one row, the nucleus taking a central position in the cell. The stroma presented no signs of activity.

HISTOCHEMICAL OBSERVATIONS:

Alkaline Phosphatase: Fig. 1

The colour reaction was present in five cases out of six. It could be seen in three sites:

1. in the glandular epithelium, at the luminal tips of the cells within the cytoplasm. The nuclei, however, showed no reaction.
2. in the surface epithelium at the free border of the cytoplasm, and in the outer cell membrane. The nuclei were also negative.
3. in the endothelial lining of blood vessels.

The results were identical in the paraffin and frozen sections.

Acid Phosphatase:

The reaction was totally absent in all the cases, in both the paraffin and the frozen sections.

Non-specific Esterase: Fig. 4

The reaction was moderately positive.

Sites:

1. In the glandular epithelium at the luminal tips of the cytoplasm.

2. At the free border of the surface epithelium in the cytoplasm.

3. In a few stromal cells scattered here and there between the glands.

The distribution was the same in both the paraffin and the frozen sections, except that in the frozen sections the reaction was present all through the tissue, while in the paraffin sections the reaction faded as we went into the depth of the tissue - Fig. 4.

Glycogen: Fig. 6

Glycogen was present, in very small amounts but detectable, in five of the six cases examined. The one which was completely negative also showed absence of alkaline phosphatase.

Location:

Glycogen granules were present at the basal parts of glandular epithelium.

Mucin: Fig. 7

Mucin was found in scanty amounts at the luminal tips of the glands in four cases out of six - Fig. 7. One case was completely negative and one showed more mucin than the others - 420/57.

Neutral Fat: Fig. 13

Small sudanophilic fat droplets were present in all the cases scattered in the stroma. Occasional

glands showed minute droplets.

Cholesterol Related Fat:

(a) Schultz Test: The blue-green colour was hardly detectable in the site of sudanophilic fat.

(b) Polarised Light: Very few doubly refractile crystals could be spotted in the fields of fat distribution. There was no significant change after treating some sections with digitonin.

(c) Sudan IV after Digitonin: No change in the sudanophilic property of the scanty fat present in the sections.

Ribonucleoprotein:

The glandular epithelium showed a moderate degree of cytoplasmic basophilia, which mostly disappeared after treatment with Ribonuclease.

II. Late Proliferative Endometrium

The number of cases which showed late proliferative histology was fifteen. Nine of them were of the persistent proliferative type; according to the dates, they were expected to show secretory changes. Six of the nine were cases of sterility and will be dealt with separately, and three were cases of upset menstruation at the age of the menopause.

Two cases showed chronic nonspecific infection. The remaining five cases showed healthy normal late proliferative histology corresponding to the dates and these will be considered now.

Histology: (From the haematoxylin and eosin preparation).

The glands were large and even tortuous. Mitosis and pseudostratification of the nuclei in glands was marked. The stroma was more active than in the early proliferative phase.

Alkaline Phosphatase: Fig. 2

This enzyme was found in larger amounts than has been noticed in the early proliferative endometrium. The distribution was the same, though the reaction was more intense and in the glands it was also seen in their lumena.

The paraffin sections showed also nuclear staining and the reaction as a whole was exaggerated. This was the finding in four out of the five patients. The fifth patient aged 34 years with irregular bleeding due to fibroids - and who had only one child - showed traces of alkaline phosphatase in both the paraffin and the frozen sections.

Acid Phosphatase:

A. Frozen sections: Fig. 3

Traces of the enzyme could be seen at the luminal tips of glandular epithelium in the cytoplasm. It was also present in the surface epithelium at the free border of the cytoplasm.

B. Paraffin sections:

The colour reaction was absent in all the cases except one where traces could be detected in the slide incubated at the higher pH 5.2.

Nonspecific Esterase: Fig. 5

The reaction was strongly positive even in the case which showed traces of alkaline phosphatase.

Sites:

1. In the glands within the cytoplasm, being denser near the lumen.
2. In the surface epithelium, also in the cytoplasm.
3. In the stroma, the colour was noticed here and there in some cells.

Glycogen:

Glycogen was present in the glandular epithelium in four cases. The amount varied from small to slight to traces. One case was negative for glycogen.

Mucin:

Mucin was present in all the cases examined at the luminal tips of the glandular epithelium and was also in

some glands within the lumena.

Neutral Fat:

Sudanophilic fat as globules could be detected under the low power. Under the high power fine granular droplets were seen in the stroma and in the epithelium of some glands.

Cholesterol Related Fat:

(a) Schultz Test: A somewhat detectable blue-green colour was noticed in the areas of sudanophilic fat distribution. The colour though detectable by the microscope was too faint to be registered with the photographic plate.

(b) Polarised Light: Doubly refractile crystals could be seen with the polarised light in the areas of fat distribution. There was no change in the shape or distribution of these birefringent crystals after treatment of some sections with digitonin.

(c) Sudan IV after Digitonin: No change in the amount nor in the distribution of the sudanophilic fat after treatment of sections with digitonin solution.

Ribonucleoprotein: Fig. 9

Cytoplasmic basophilia is marked in this period of maximal proliferation.

III. Early Secretory Endometrium

Six cases of early secretory endometrium were studied. They were free from inflammation and the dates corresponded more or less with the histological picture.

Histology:

The glands showed the characteristic subnuclear vacuolation; they were not yet as tortuous as in the fluorescent secretory phase. The stromal activity was just beginning to be manifest by nuclear mitosis.

Alkaline Phosphatase: The reaction is evidently less than in the proliferative phase. The amount varies from moderate to slight.

Location:

1. In the glands it is seen at the very tips of glandular epithelium or within the lumens of some glands.
2. In the surface epithelium at the outer border of the cells.
3. In the endothelium of blood vessels.

Acid Phosphatase:

The reaction is evidently more than in the proliferative phase. The amount is moderate. This was constant in all the frozen sections. In the paraffin sections, the reaction was less and in some slides absent. The distribution

of the enzyme in the endometrium in this phase was in the cytoplasm of glandular and surface epithelium. The concentration is more towards the lumens of glands and free border of the surface epithelium. Some of the paraffin sections with the Gomori method gave only a nuclear reaction.

Nonspecific Esterase:

In the four cases where it had been performed, moderate amounts were encountered in all the frozen sections.

The amounts varied in the acetone-fixed tissues but the reaction was always present.

The distribution was in the cytoplasm of the glandular and surface epithelium, the reaction being more intense at the luminal tips of the glandular epithelium. Some cells in the stroma showed a colour reaction in the whole or in part of the cell. The nuclei of the glandular and surface epithelium were devoid of the colour reaction.

Glycogen:

Glycogen was present in all the cases both with the chromic acid Schiff and the Best's stain.

Location:

In all the cases, glycogen was present in the glandular epithelium, at the basal part. In the surface epithelium it was also present in the stromal cells as fine granules.

Mucin:

Mucin was seen in all the cases in the lumens of glands.

Neutral Fat:

Under the low power some sudanophilic globules could be seen. Using the high power of the microscope, fine granules were seen in the stroma.

Cholesterol Related Fat:

(a) Schultz Test: A blue-green colour could be seen in the areas of sudanophilic fat. The intensity of the colour was still not strong enough to be photographed.

(b) Polarised Light: Doubly refractile crystals were encountered in the areas of fat distribution. The amount and shape of birefringent material was not changed by digitonin treatment of the section.

(c) Sudan IV after Digitonin: There was no change in the amount of sudanophilic material, hence this fatty substance is not free cholesterol.

Ribonucleoprotein: Fig. 12

The intensity of cytoplasmic basophilia in the glandular epithelium was less than what was seen in the late proliferative phase.

IV. Mid-Secretory Endometrium

Fifteen cases of mid-secretory histology were available for study. The endometrium was healthy and the histologic picture conformed with the corresponding dates of the menstrual cycle.

Histology:

The glands showed the characteristic saw-teeth appearance. The nuclei of the glandular epithelium were basal in position, and the luminal tips of the cytoplasm looked just melting into secretion.

Alkaline Phosphatase: Fig. 17.

In twelve cases the distribution was as follows:

In the glands scanty amounts were noticed in the tips of the epithelium or lumena of some glands. Other glands were negative.

In the surface epithelium it was almost negative.

In the blood vessels the lining of the vessels was constantly positive. In three cases the reaction was manifest only in the lining of blood vessels.

Observations were identical in paraffin and frozen sections.

Acid Phosphatase:

Frozen Azo Method: Fig. 19

In the nine cases where it was performed, the intensity of the reaction was moderate in eight and

slight in one case where the patient was forty-seven years old. The reaction was seen in the cytoplasm of glandular and surface epithelium. It was also noticed in the secretion of some glands within the lumena.

Gomori Paraffin Method: Fig. 20

The slides were either completely negative at both pH 4.8 and 5.2 or showed a nuclear reaction. The nuclear reaction presented in eight of the fourteen cases investigated. The site was chiefly in the stroma in patchy distribution. It was more marked at the lower pH.

Non-specific Esterase: Fig. 21

This enzyme was investigated in the most recent six cases. In all of them the reaction was present, but the intensity was less than that seen in the proliferative phase. These findings were the same in both the frozen Azo and the Paraffin Azo sections.

Location:

The colour indicating the activity of this enzyme was present in the glandular and surface epithelium, in the cytoplasm at the inner and outer borders of the cells; the nuclei were colour-free. Some deposits of the coloured Azo compound were noticed in scattered cells in the stroma.

Glycogen: Figs. 23 and 24

In all the cases, whether treated with the chromic acid Schiff or Best's carmine stain, the findings were nearly the same and the distribution of glycogen in the endometrium was as follows:

In the stroma: very fine granules of glycogen were present in the stroma cells.

In the surface epithelium: glycogen granules of moderate size were encountered in parts of the surface epithelium at the basal part of the cells. Glycogen was weakly positive in two cases, one aged 47 and the other aged 39.

Mucin: Fig. 22

In all the cases mucin was present in large amounts inside the lumena of glands. A strip of mucin could be detected at the outer margin of the surface epithelium (e.g. case 3315/57).

Neutral Fat: Fig. 14

Sudanophilic fat granules were present in the stroma of the superficial layer of the endometrium. Fat granules could also be seen in the glandular epithelium in some areas.

Cholesterol Related Fat:

(a) Schultz Reaction: a blue-green colour, not very bright, and weak, was present in the site of the sudanophilic fat.

(b) Polarised Light: Fig, 15. Some doubly refractile crystals are present in the site of fat in the endometrium. No change after digitonin.

(c) Digitonin Precipitation: Staining with Sudan IV after treating some sections with the digitonin solution revealed no significant change in the amount of sudanophilic fat.

Ribonucleoprotein:

Cytoplasmic basophilia was still less than in the preceding phases of normal endometrium.

V. Late Secretory Endometrium

Six cases in this category were available.

Histology:

The glands coming to the end of their duty seem to involute back to a smaller size, their epithelium tending to be cuboidal with the nuclei coming back to the centre of the cells.

The remains of the secretions could be seen in the lumens of some glands.

The histochemical reactions seemed to be just a continuation of what was noticed in the mid-secretory phase.

All the substances investigated were increased except alkaline phosphatase and ribonucleoprotein (Figs. 10 and 11). The alkaline phosphatase was fading away; only occasionally

could it be traced in the lumen of a gland. The endothelium of blood vessels still shows a colour reaction (Fig. 18).

B. PATHOLOGICAL ENDOMETRIUM

I. Anovulatory Endometrium in Sterility

Curettings from six cases of primary sterility were examined. As the amount of tissue sent in these cases was always scanty (premenstrual biopsy), only a limited number of reactions could be carried out.

The histology from the haematoxylin and eosin preparation showed a persistently proliferative type of endometrium.

Alkaline Phosphatase:

This enzyme was greatly diminished than in the normal endometrium of the proliferative phase.

Acid Phosphatase:

This was either absent or present only in minute traces.

Glycogen:

This was either present in small amounts or absent.

Neutral Fat:

This was scanty or absent.

II. Menopausal Endometrium

Three cases of irregular menstruation about menopausal age were available. The last menstrual period before curettage was twenty days, two months, and thirty-seven days in the three cases.

The histological picture of the endometrium was rather atrophic in the three cases.

Histochemical Features:

Alkaline Phosphatase:

Traces of the colour reaction could be seen in both the frozen and the paraffin sections. In one of the three cases (a hysterectomy specimen) the zona basalis of the endometrium was completely devoid of alkaline phosphatase.

Acid Phosphatase:

The colour reaction was present in faint traces in two cases. In one case the amount was the same as in a normal late proliferative endometrium in some glands which looked actively proliferating and in traces in the glands which were in the resting non-proliferating condition.

Ribonucleoprotein:

Cytoplasmic basophilia could hardly be seen in the cytoplasm of the glandular epithelium.

Non-specific Esterase:

The reaction was as intense as that in a normal late proliferative endometrium.

Glycogen:

In one case glycogen was absent altogether. In the other two cases, glycogen was present in slight amounts in the glandular and in the surface epithelium, but not in the stroma.

Neutral Fat:

Very fine sudanophilic fat droplets were present in some areas of the endometrium in one case. In the other two cases, fat was almost absent.

III. Non-specific Inflammations of the Endometrium

A. Infected Endometrium in the Proliferative Phase (2 cases)

Significant Histochemical Changes:

1. Alkaline phosphatase was more increased than in a normal proliferative endometrium. It could also be seen in the inflammatory cells.
2. Acid phosphatase by the paraffin method showed an intense nuclear reaction in both glands and stroma.

B. Infected Endometrium in the Secretory Phase (2 cases)

One of the two cases seemed to have suffered a more severe and long standing infection than the

other. This is evidenced by the fact that blood vessels in the granulation tissue were thicker and more numerous and by the heavy round cell infiltration.

Observations:

In the heavily and more chronically infected endometrium, there was marked increase in the amounts of alkaline and acid phosphatase. There was also an increase in the amount of mucin, especially in the areas of infiltration. The surface epithelium over the infected area showed marked staining for mucin in the whole cytoplasm.

In the case with mild infection, no appreciable change was noticed.

IV. Cystic Glandular Hyperplasia of the Endometrium (Metropathia Haemorrhagica)

Twelve cases of cystic glandular hyperplasia of the endometrium were available for histochemical examination. The history was typical of metropathia haemorrhagica with periods of amenorrhoea followed by bleeding.

Histology:

The endometrium in these cases had the full-blown picture of cystic glandular hyperplasia with marked disparity in the gland pattern. Some glands were large and cystic with one layer of epithelium and a central nucleus; others were small and sometimes showed stratification or

pseudostratification of the nuclei. The nuclei were deeply stained with haematoxylin and frequently showed mitotic figures. The stroma was dense, abundant and sometimes showed equally active mitoses. The surface epithelium was taller than normal.

Alkaline Phosphatase: Fig. 25

This enzyme was markedly increased and even exaggerated by the paraffin method.

Acid Phosphatase:

By the Azo Frozen Method: (Fig. 26) this enzyme was only present in mere traces at the tip region of the glandular epithelium and sometimes was totally absent.

By the Gomori Paraffin Method: the enzyme was either absent or a nuclear reaction might be there in the stroma. This nuclear reaction occurred at the lower pH 4.8.

Non-specific Esterase: Fig. 27

This enzyme was present in appreciable amounts, in the glandular epithelium, surface epithelium and scattered in the stroma. The reaction by the azo paraffin method showed somewhat smaller amounts than those seen in the frozen sections.

Glycogen: Fig. 29

The amounts of glycogen seen by both stains were scanty and in some cases glycogen could not be detected in the sections. These small amounts were encountered

at the bases of some glandular epithelial cells or in a few areas of the surface epithelium.

Mucin: Fig. 30

Mucin was present in almost all the glands. Most of the cystic glands were full of mucin in their lumena while the glandular epithelium itself might show the characteristic red colour with the mucicarmine stain in the cytoplasm.

Neutral Fat: Fig. 31

Marked increase in the sudanophilic fat droplets was noticed in all the cases. Fat was located in the stroma and the glands were conspicuously devoid of fat.

Cholesterol Related Fat:

(a) Schultz Test: (Fig. 33). The characteristic blue-green colour was seen in the stroma in places where sudanophilic fat was present. The colour was quite evident and showed well in the photograph.

(b) Polarised Light: (Fig. 32). Birefringent crystals were evident under polarised light; they were located in the stroma in between the shadows of the cystic glands. There was no change in the site, size, or shape of the birefringent crystals after digitonin.

(c) Digitonin Precipitation before Sudan Staining: Staining with Sudan IV after incubation of some frozen sections with 0.5% digitonin solution in 50% alcohol gave the following result: no appreciable change in the amount or distribution of the sudanophilic fat.

Ribonucleoprotein: Fig. 28

Cytoplasmic basophilia was more intense than was noticed in the normal endometrium. This was quite evident in the glandular epithelium.

V. Endometrial Carcinoma

Recently much interest has been aroused in the enzymic activity of malignant tissues. One of the enzymes considered significant in relation to cancer, especially of the genital system, is glucuronidase. This enzyme has been found to be increased in cancer of the cervix, of the vagina and of the vulva. In the endometrium, however, biochemical assays showed no increase of glucuronidase activity in endometrial cancer (Lorincz and associates, 1951; Wied and Sechelman, 1952).

No attempt, therefore, was made in this study to investigate glucuronidase activity of the endometrium by histochemical methods.

The previously mentioned seven substances were investigated histochemically in ten cases of fundal carcinoma of the uterus.

The specimens were obtained from uteri just after their removal at operation in cases which were proven to be cancer by a previous curettage.

One case of endometrial cancer which had received radium for one week before the operation was also available for study.

Histology:

All endometrial cancers in this study were of the adenocarcinomatous variety. Most of them were papillary. There was a certain degree of adenocanthosis in one case.

Alkaline Phosphatase:

By the Frozen Azo Method: (Fig. 34) the reaction was totally absent from the adenocarcinomatous tissue except in the blood vessels in three of the ten cases. The remaining seven cases showed marked diminution of the intensity of the colour, and the distribution in the carcinomatous glands was patchy. The blood vessels were always positive.

By the Paraffin Method of Gomori: (Fig. 35) only two cases showed apparently normal amounts of alkaline phosphatase, taking the distribution as that of a normal healthy actively proliferating endometrium. In the other eight cases the amounts were either small, scanty or

absent from cancerous tissue except in the blood vessel endothelium.

Acid Phosphatase:

By the Frozen Azo Method: (Fig. 36) in all the specimens the reaction was present in appreciable intensity, exceeding that in a normal endometrium even in the secretory phase. The site was the cytoplasm of the glandular and surface epithelium.

By the Paraffin Method of Gomori: (Fig. 37) the reaction was present in all the cases studied. In most of them it was of a severe intensity, but in some it was patchy in distribution with areas showing a stronger reaction than the remaining cancerous tissue. The colour was quite evident in the nuclei of both the glandular and stromal elements. It was also seen in the lumena and luminal border of some glands.

Non-specific Esterase: Figs. 38 and 39

A marked increase in the activity of this enzyme was noticed in all the cases, the paraffin sections showing lesser amounts than the frozen ones.

Sites:

1. In the glandular epithelium at the inner and outer borders of the nuclei.
2. In the surface epithelium, being heavier at the free border.
3. In the stroma scattered in certain cells.

Glycogen:

Glycogen was present in large amounts in the glandular epithelium in two of the cases examined. It was absent in four cases and was present in traces in four. The site of glycogen when present was the cytoplasm of glandular or surface epithelium.

The marked increase in glycogen content in one case was associated with invasion of the uterine muscle with the cancerous growth (Figs. 40 and 41).

Mucin: Fig. 43

Large amounts of mucin were noticed in the lumena of cystic glands; otherwise mucin was present in amounts similar to those seen in a normal endometrium. In one case the glandular epithelium itself picked up the red mucicarmine stain in its cytoplasm. In this particular case, areas of mucigenic epithelium like that of the cervix were scattered in the field of carcinomatous growth.

Neutral Fat: Fig. 44

It was a striking feature of all the cases examined of cancer endometrium that they showed marked increase in the amount of sudanophilic material. The size of fat globules was big and the site was in the stroma between the glands.

Cholesterol Related Fat:

(a) Schultz Test: (Fig. 45). The most intense blue-green colour given with this test was seen

in cases of carcinoma, its site being in the stroma. There was also a positive reaction in the cancerous epithelium in a few glands at the inner and outer borders of the epithelium and sometimes inside the lumena. In the areas of necrotising cancerous tissue, the green colour was absent.

(b) Polarised Light: (Figs. 46 and 47). Doubly refractile crystals were present in all the sites of fat distribution, and not only in the areas giving a positive Schultz test. The birefringent crystals were either needle-shaped, clusters or dots. There was one case which showed marked invasion of the uterine muscle with the cancerous growth. The invaded muscle showed plenty of sudanophilic fat. This fat under polarised light showed many crystals, doubly refractile with the Maltese cross pattern all over the invaded uterine wall.

(c) Sudan IV after Digitonin: There was no change in the sudanophilic property of the fat present in living cancerous growth, indicating that this fat is not free cholesterol.

Ribonucleoprotein: Fig. 42

Cytoplasmic basophilia was heavier than ever seen in the endometrium in all the previous conditions. This

basophilia was absent in the control slide incubated with Ribonuclease solution.

A Case of Cancer Endometrium with Early Effects of Irradiation

This was a case of adenocarcinoma of the endometrium who had received radium for one week before the operation.

Histology:

Early signs of irradiation were indicated by the presence of inflammatory cells, mostly lymphocytes and eosinophils. Very few cells of the cancerous tissue showed early signs of degeneration. Karyorrhexis and karyolysis in the nuclei were not yet developed.

Histochemical Observations:

The significant differences between this case and the other cases of endometrial cancer examined, were as follows:

Alkaline Phosphatase: Fig. 48

Enormous amounts of alkaline phosphatase were present in the cancerous epithelium as well as in the blood vessels of the uterine wall.

Acid Phosphatase: Fig. 49

No increase in the activity of this enzyme was noticed in the irradiated cancerous tissue.

Mucin: Fig. 50

Mucin was present in large amounts inside the lumena of dilated glands and in collections of inflammatory cells.

D I S C U S S I O N

THE NORMAL HUMAN ENDOMETRIUM

From the preceding observations we see that in the normal human endometrium alkaline phosphatase and ribonucleoprotein preponderate during the proliferative phase, while glycogen, acid phosphatase, fat and mucin, increase during the secretory phase.

Distribution in Endometrial Tissues:-

All these substances were present in the epithelial elements of the endometrium, in the glandular and in the surface epithelium.

Fat had its primary seat in the stroma cells, and a small proportion of the glands showed fat in their cells or lumena.

Non-specific esterase was found in a few cells scattered here and there in between the glands, while the glandular and surface epithelium showed strong activity of this enzyme.

Phosphatase, ribonucleoprotein and mucin were not seen in the stroma cells in the normal non-pregnant endometrium.

Alkaline phosphatase, besides its activity shown by the epithelium, was constantly seen in the endothelium of the endometrial blood vessels.

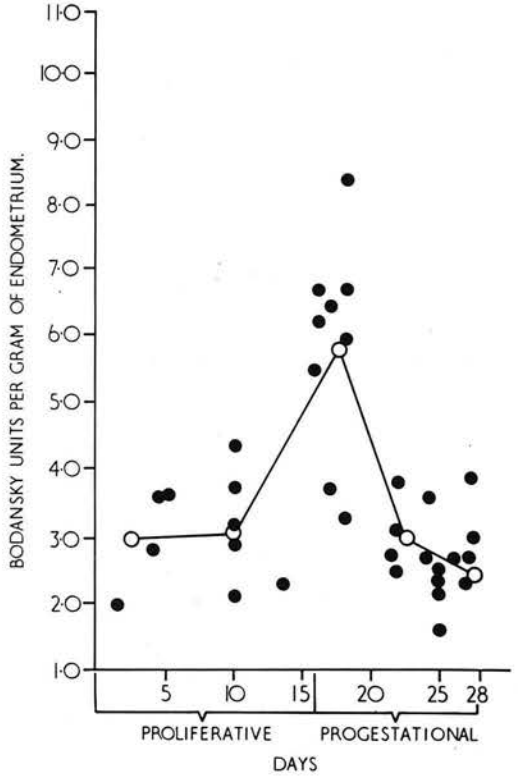
All these substances were present in the cytoplasm. The nuclear reaction given by the Calcium-Cobalt reaction and lead phosphate method for alkaline and acid phosphatases respectively, is doubtful, since no such reaction is shown by the Azo-coupling methods in frozen sections.

Alkaline and Acid Phosphatase in the Normal Endometrium

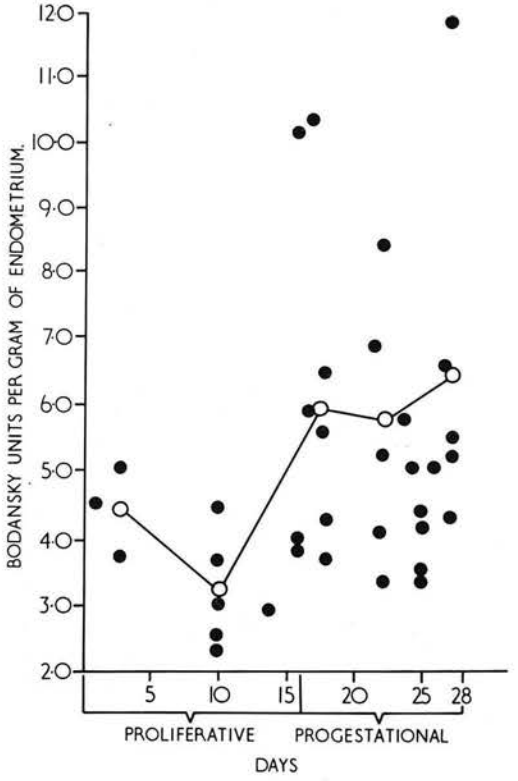
The observations in this study, confirm the recent work of McKay et al. (1956) in a similar histochemical study of the human endometrium.

They carried out a quantitative determination of endometrial phosphatases by chemical means, and established agreement between chemical and histochemical results concerning these phosphatases. They illustrated the results of their chemical estimations by two curves which are worthy of presentation here:

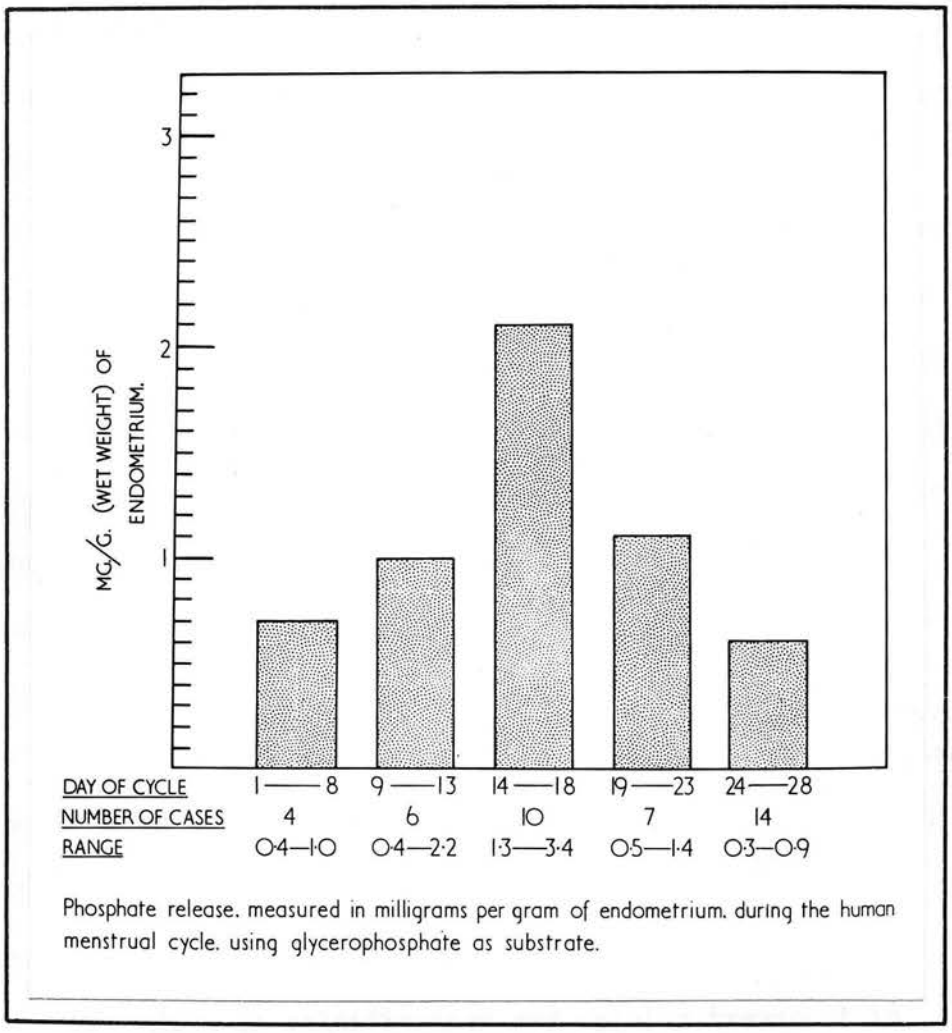
Quantitative variation in endometrial alkaline phosphatase activity during the menstrual cycle.



Quantitative variation in endometrial acid phosphatase activity during the menstrual cycle.



Jones and associates in 1952 carried out a biochemical and a histochemical study of alkaline phosphatase in forty-one cases of normal cycles and found similar results. They illustrated their biochemical results in the following diagram.



Phosphate release. measured in milligrams per gram of endometrium. during the human menstrual cycle. using glycerophosphate as substrate.

Alkaline Phosphatase and its Role in the Normal Endometrium

Alkaline phosphatase activity starts in the epithelial elements of the endometrium with the beginning of proliferation. It increases as proliferation advances, but with the onset of secretory activity the amount of alkaline phosphatase in the epithelial elements suddenly drops, and by the end of the cycle the glandular and surface epithelium is depleted.

Some alkaline phosphatase is seen within the lumena of glands in the secretory phase. We cannot attribute any function to this alkaline phosphatase seen with other secretions. As the substances secreted by the endometrium are supposed to be ready for utilisation by the young fertilised ovum, which already has a high alkaline phosphatase activity of its implanting trophoblast (as will be seen in the second part of the study concerning the placenta), the alkaline phosphatase in the secretion can be regarded just as residual from epithelial activity prior to secretion.

The function of alkaline phosphatase of the endometrium is not yet well understood. Moog (1946) and Pearse (1953) summarised the functions of alkaline phosphatase in the human body thus:

1. Role in calcification and calcium transport in the body.
2. Absorption of glucose in renal tubules and intestines.

3. Production of milk in the mammary gland.

Essentially, phosphatases are hydrolysing enzymes, capable of catalysing reversible reactions of phosphate esters. They can therefore be involved in carbohydrate metabolism, nucleotide metabolism, phospholipid metabolism and calcium metabolism (Sumner and Somers, 1943 & 1953).

Relation of Alkaline Phosphatase to Protein Synthesis:

The simultaneous presence of alkaline phosphatase with ribonucleoprotein during the proliferative phase - the phase of growth of the endometrium - suggests that this enzyme is concerned with protein synthesis.

Casperson (1947), in an extensive review, presented considerable evidence that cytoplasmic ribonucleic acid plays an indispensable role in the synthesis of protein, and that the ribonucleic acid content in the cytoplasm of a cell provides an index of the rate of protein synthesis.

Stein and Stuermer in 1951 showed that the ribonucleic acid concent of the endometrium could be regarded as an index to the rate of protein synthesis. Dempsey and Wislocki (1946) in a "Histochemical Contribution to Physiology", concluded that alkaline phosphatase helps in the building up of proteins, as it occurs in abundance with nucleoproteins in the endometrial epithelium during the proliferative phase.

Moog (1946), reckoned the common finding of phosphatase in the cytoplasm of growing, regenerating and secreting cells

in which protein synthesis is being carried out.

Meyer and McShan (1950), from their studies on ovarian tissues in rats and mice, arrived at the conclusion that alkaline phosphatase is cytoplasmic, and increases with increase in the size of the cells. On the other hand, Atkinson et al. (1949), in a study of ribonucleic acid in normal and pathological glandular epithelium, pointed out the significance of the relation of alkaline phosphatase to protein synthesis to be entirely speculative.

Hormonal Relations:

The occurrence of alkaline phosphatase and ribonucleoprotein together in the proliferative phase of the cycle leads to the assumption that their presence is the result of oestrogenic activity. This assumption has been discussed by many authors who reported similar findings.

Experiments on ovariectomised animals, treated with oestrogen and with progesterone, showed that oestrogen stimulated while progesterone inhibited endometrial alkaline phosphatase.

Atkinson and Elftman (1946), Veraldo (1954), and Atkinson and Engle (1947), experimenting on mice, rats and monkeys respectively, confirmed the assumption. Atkinson (1950), reported on the cyclic variation of alkaline phosphatase in the human endometrium, and mentioned its

absence from the epithelium in three anovulatory cycles.

Hall and Brooklyn (1950) also described the cyclic variation in the human endometrium.

Telfer demonstrated that injection of oestrogen into ovariectomised rats caused increased production of ribonucleoprotein by the rat uterus.

There is no doubt, therefore, that a true relation exists between oestrogens and these two substances in the endometrium.

Relation of Alkaline Phosphatase to Glycogen Formation:

Wislocki and Dempsey (1945), in their study of pregnant endometria in women and in animals, emphasised that in spite of a range of anatomical variation of the location of the enzyme in the different species, alkaline phosphatase was constantly present at some site between maternal blood in the endometrial capillaries, and the site of glycogen deposition.

Arazac and Blanchet (1948) also believed that alkaline phosphatase activity in the endometrium had to precede glycogen formation.

It would appear, however, that alkaline phosphatase of the capillary endothelium in the endometrium is concerned with absorption of glucose from the maternal blood, a similar role to alkaline phosphatase in intestinal mucosa and renal tubules; but regarding the deposition of

glycogen in the glandular epithelium and stroma cells, one cannot be sure of the role of alkaline phosphatase. Alkaline phosphatase is never seen in the stroma cells, while glycogen is normally present in them in the secretory phase, and in abundance when they become decidual cells in early pregnancy. In the epithelial cells, alkaline phosphatase is usually at the outer border of the cytoplasm, while glycogen starts to show at the basal (subnuclear) part. Moreover, in hyperplasia of the endometrium, alkaline phosphatase is abundant in the epithelium, while glycogen is scanty or absent.

Glycogen in the Normal Endometrium:

We have seen that glycogen is present in small amounts in the proliferative phase and increases remarkably in the secretory phase. There is a fairly general agreement to this in all previous studies, some of which correlated histochemically with quantitative chemical estimations. The literature pertaining to this subject over the period from 1910 to 1946 has been extensively reviewed by Reynold (1949).

Among more recent reports on endometrial glycogen are those by Hagerman and Vilee (1953), Skjoldberg (1954) and McKay (1956).

Hagerman and Vilee (1953) studied the effect of oestrogens on the metabolism of endometrial tissues in vivo and in vitro. They found that oestradiol stimulates the

metabolism of endometrial tissues, favouring the breakdown of carbohydrates, as in the Krebs cycle, towards the production of CO_2 and H_2O.

Skjoldberg (1954) studied endometrial glycogen histochemically and by chemical estimation. He found that endometrial glycogen amounts to 10-30 mgm. per cent in the proliferative phase, and 45-80 mgm. per cent in the secretory phase.

McKay (1956) made a histochemical study, and his results agree with those of other workers.

Effects of Ovarian Hormones on Endometrial Glycogen:

Experiments on animals have been carried out to verify the role of ovarian hormones in the deposition of endometrial glycogen.

Hisaw (1935) found that the richest accumulation of glycogen occurs under the influence of progesterone at the time of greater glandular hypertrophy late in the cycle. Overhelser and Nelson (1936) experimenting on monkeys and rats found that oestrogen treatment over a long period resulted in the deposition of large amounts of glycogen in the mucosa of the corpus uteri.

Skjoldberg (1954) injected oestrogen during the proliferative phase followed by progesterone during the secretory phase to spayed female animals as well as to sterile women showing no glycogen in the endometrium. He

concluded that the total amount of glycogen in the endometrium depends on the action of oestrogen, whereas the site depends on the action of progesterone.

That oestrogens alone when given over a long period can lead to glycogen deposition in the endometrium does not comply with the fact that glycogen is scanty in cystic glandular hyperplasia, which is considered a condition of hyperoestrinism

In the light of the present knowledge, however, it can be summarised that glycogen in the endometrium is a result of progestational effect provided the oestrogenic influence on the endometrium is adequate.

Acid Phosphatase in the Normal Endometrium:

In the secretory phase there is a remarkable increase in acid phosphatase, the action of which, according to Sumner and Somers (1953) is essentially to hydrolyse the same mono-esters of phosphoric acid as are hydrolysed by alkaline phosphatase.

We have assumed that alkaline phosphatase is concerned with phosphorylation and absorption of glucose from the maternal blood in the endometrial capillaries. One would therefore also assume that acid phosphatase is concerned firstly with the pre-ultimate dephosphorylation at the site of glycogen deposition and secondly with its secretion through the free border of the cells. Acid phosphatase

can be seen in the stroma cells especially when they become transformed into decidual cells, which are a favourite site for glycogen deposition. A further proof of the role of acid phosphatase in glycogen deposition might be in the fact that in cystic glandular hyperplasia of the endometrium both substances are present in small amounts.

In endometrial cancer, the correlation between the two substances is not constant, but cancer does not appear to conform to the rules governing normal physiology.

The optimum pH for acid phosphatase is 4-6. Stein and Stuermer (1950), studying the effect of pH on the endometrium in tissue cultures, found that proliferative endometrium is best kept at pH slightly above 7 (7.3-7.6), and that secretory endometrium is best kept at pH slightly below 7 (6.7-6.9). It is logical then to find increased activity of alkaline phosphatase in the proliferative phase and of acid phosphatase in the secretory phase. This leads us to think of an ionic mechanism, most probably governed by hormones, regulating the pH of the endometrium for normal function. Corner and Csapo (1953), studying the action of ovarian hormones on uterine muscle, concluded that hormonal effects might be exerted primarily upon the ionic balance of the living cell.

Fat in the Normal Endometrium:

During the whole menstrual cycle fat is present in

the stroma and gradually increases in amount towards the end of the cycle. It is more abundant in the secretory than in the proliferative phase. The glandular and surface epithelium, during the proliferative and early secretory phase, contains very little fat. In the mid and late secretory phase, there is markedly more fat in the epithelial cells, first in their basal part, then in the marginal part and in the secretion.

Fat is more abundant in the superficial part of the endometrium. The distribution of fat in the normal human endometrium in the various phases of the cycle, as well as in the first half of pregnancy, has been adequately studied by Gillman (1940 and 1941). He related the basal epithelial fat to the effect of progesterone, and recommended that it could be used as a test for the physiological activity of progesterone in endometria of the late second half of the cycle. Moreover, he noticed in pregnant endometria that fat in the decidua reached a peak at about the 70th day of pregnancy, and by that time the corpus luteum, according to Gillman and Stein (1941), has just passed its maximal development (around the 60th day).

Gilbert (1942), experimenting on castrated rabbits, found that in endometria previously synthetised by oestradiol benzoate, combinations of oestradiol benzoate with adequate amounts of progesterone, which are associated

with progestational response, cause the appearance of abundant fat in the infranuclear region of the glandular epithelial cells and in the stromal cells.

We can therefore postulate that the basal fat in the epithelial cells of the endometrium when present in adequate amounts implies a good hormonal balance in a reacting endometrium.

Wislocki and Dempsey (1945), in their study of pregnant endometria, found that in the human uterus during the early months of pregnancy, sudanophilic fat was abundantly present in the glandular and surface epithelium and, to a less and variable extent, in some of the decidual cells.

The Nature of Fat in the Endometrium:

The occurrence of lipoid in the epithelium and stroma of the endometrium was previously regarded as a manifestation of degeneration (Froboese, 1924; Bartelmez and Bensley, 1932; Rossman, 1941). More recently Gillman (1941) and Gilbert (1942) have questioned this assumption in man and in rabbit respectively, pointing out that much of the fat is situated basally in the cells, and that its presence there is regulated by ovarian hormones.

In this study the endometrial fat was found to give a positive Schultz test, which was weak in the proliferative and more evident in the secretory phase.

Significance of the Schultz Test:

This test, which is an adaptation to histology of the Liebermann-Burchardt sterol reaction, is considered the most specific test for cholesterol and its esters. The Liebermann-Burchardt reaction (cf. Pearse) has been considered by Bierry and Gouzon (1936) to be a general reaction of unsaturated steroids, and by Everett (1947) to indicate the presence of diols formed by mild oxidative processes, while Boscott and Mandl (1949) applied the reaction to pure samples of dehydro-iso-androsterone, progesterone and deoxycorticosterone acetate, without obtaining the characteristic blue-green colour, but instead they obtained colours varying from yellow to orange.

In this study, endometrial fat gave the characteristic blue-green colour, the intensity of which increased with the increase in the amount of fat. This means that it has a steroid structure related to that of cholesterol.

Windaws Digitonin Reaction:

This reaction was carried out to know whether the Schultz positive fat is cholesterol itself or something of a related structure. According to the results of this test, endometrial fat is not free cholesterol. Therefore it must be either an ester of cholesterol or another substance possessing the steroid ring of cholesterol.

Birefringence:

We have seen that some doubly refractile crystals were present in the sites of fat distribution in the endometrium. Wislocki and Dempsey (1945) failed to show birefringence of lipoidal inclusions of the endometria of sow, cat, rat and woman.

In 1948, Wislocki and Dempsey again reported that fat droplets in the epithelium of uterine glands and in decidual cells were not birefringent.

Significance of Birefringence:

Neutral fats ordinarily remain dark when examined in the dark field produced by crossing the Nicol prisms, though any fatty substance in solid crystalline form may be luminous under polarised light.

Free cholesterol is seen as long rhomboidal crystals which glow under polarised light, and are extinguished and light up alternately once in each 90° of rotation of the stage.

Substances forming Lehmann's "Liquid Crystals" such as cholesteryl esters, phosphatides and cerebrosides, may exhibit the black cross of polarisation with luminous quadrants between the arms of the cross filling out a circle (Maltese Cross). This phenomenon is suppressed if the temperature is above that at which the liquid crystals in question can exist and the globules remain dark.

Birefringence alone cannot be relied upon for the identification of the type of fat, but the fact that endometrial fat gives a positive Schultz test and shows birefringence, which in part is of the Maltese Cross type, still emphasises that endometrial fat is of a steroid structure, and still we cannot precisely say what exactly this fatty substance is.

Since endometrial fat gives positive steroid reactions and shows cyclic variations which follow the ovarian hormones, there is a possibility that endometrial fat represents a hormone, the role of which is not fully understood.

The search for a uterine hormone was carried out by different investigators:

Sessums and Murphy in 1933 described follicle degeneration and inhibition of new follicle growth in hysterectomised rabbits, which could be prevented by implanting their endometrium in the rectus sheath. Cheval in 1934, experimenting on dogs, obtained similar results. Mishel and Motyloff in 1941 treated ovarian atrophy in hysterectomised rabbits by an extract from the cow's endometrium. Tenny, Parker and Robbins in 1955 described the changes in the ovaries and adrenals of hysterectomised rabbits, and in 1958 they found the aqueous extract of the human uterus effective against ovarian atrophy in hysterectomised rabbits.

Whether endometrial fat has anything to do with this particular substance involved in the endocrine system is a matter that needs further investigation, chemically, histochemically and by using such physical characters of steroids as autoflourescence, etc.

For the investigation of hormones the histochemical field is a rather limited one. Attempts to identify and localise hormones in the tissues by histochemical methods are all indirect, and conclusions from available evidence are only presumptive. An example of these efforts is the study of ketosteroids in the adrenal cortex of cats by Bennet (1940). Lipoids in the ovary were investigated on the same lines as Bennet's, by Dempsey and Basset in 1943 (rat ovary) and by McKay and Robinson in 1947 (human ovary). On the same lines also, lipoids in the human placenta were studied by Dempsey and Wislocki in 1944.

In 1945 Wislocki and Dempsey applied the plasmal Schiff reagent to pregnant endometria, and reported on a positive Schiff reaction in epithelial cells, which was abolished on treatment of the sections with acetone and alcohol. They concluded that such fat in endometrial epithelium might be of a steroid structure similar to that of ovarian hormones. They did not report on the behaviour of fat in the stroma, which is the main site of endometrial fat.

Conclusions:

Fat in the endometrium was thought to be merely a

nutritive store for the ovum and embryo, the same as is glycogen, but it seems that our concept of the nature and function of endometrial fat has to be modified. There is a strong possibility that endometrial fat, at least in part, represents a steroid hormone. Further studies are required to identify the exact nature of this hormone.

Non-specific Esterase of the Endometrium

Epithelial non-specific esterase activity was found to be more in the proliferative than in the secretory phase. When secretion actually starts, it is seen in the lumena of the glands. A certain amount of activity is seen in some stroma cells all through the cycle.

The reaction thus observed may represent the activity of several specific enzymes, one of which is Lipase, which hydrolyses true fats but histochemically is difficult to isolate. Since the reaction can represent the activity of more than one specific enzyme, we cannot precisely define its physiologic role in the endometrium.

Mammalian tissues with high non-specific esterase activity are the lung, liver, pancreas and kidney (Nachlas and Seligman, 1949b, and Huggins and Moulton, 1948).

The purpose of studying this reaction in the endometrium is to know whether there is any deviation from normal in pathological conditions, especially carcinoma.

Cohen, Nachlas and Seligman (1951) reported that, except for thyroid lesions, cancers had less non-specific esterase activity than the homologous normal tissues, but acceptance of such a generalisation should be preceded by more extensive studies of cancer in individual organs.

Gross and Danziger (1957) showed by histochemical methods that untreated malignant lesions of the cervix displayed greater non-specific esterase activity than did the normal cervical epithelium. Non-specific esterase in endometrial cancer will be dealt with later.

Mucin

Mucin was seen at the free margins of the glandular and surface epithelial cells of the endometrium in the late proliferative phase. It was not seen within the depth of the cytoplasm of the epithelial cells, and it is generally assumed that mucin is formed from its precursor at the time of its secretion, and this as we have noticed takes place at the free border of the cell.

Mucus secretion precedes all other secretions which result from progestational activity.

The function of mucin in the endometrial secretion can be summarised as follows:

1. Mechanical, as lubricant and protector to spermatozoa and fertilised ovum.
2. Nutritive, (glycoprotein and mucopolysaccharide),

3. Probably concerned with maintaining an optimum pH for sperms during their journey in the uterine cavity.

SUMMARY

Substances which can be correlated to oestrogenic activity in the endometrium are:

Alkaline Phosphatase

Ribonucleoprotein

Mucin

Non-specific Esterase.

Substances which can be taken as criteria of adequate hormonal balance (oestrogen and progesterone) are:-

Acid Phosphatase

Glycogen

Lipoids.

PATHOLOGICAL ENDOMETRIUM

I. Anovulatory Endometrium in Sterility

In six cases of primary sterility, the endometrium showed persistently proliferative histology as a sign of absent ovulation. In these cases the following substances were either scanty or absent.

1. Alkaline phosphatase
2. Acid phosphatase
3. Glycogen
4. Fat.

The diminution of the last three substances is attributed to the lack of progestational influence secondary to absence of ovulation.

The strange feature is the absence of diminution of alkaline phosphatase the activity of which has been attributed to the effect of oestrogen. This can be due to:

1. a faulty endometrium not responding to ovarian oestrogen;
2. oestrogen deficiency.

II. Menopausal Endometrium

At the menopause and at the climacteric where the endometrium was rather atrophic, the following substances were present only in small amounts:

1. Alkaline phosphatase
2. Acid phosphatase

3. Glycogen

4. Fat.

This means that both the governing ovarian hormones are lacking. We can relate this picture to ovarian atrophy with consequent low levels of oestrogen and progesterone.

III. Non-specific Inflammation of the Endometrium

The changes noticed in non-specific inflammations of the endometrium are:

1. Increased alkaline phosphatase

2. Increased acid phosphatase

3. Increased mucous secretion.

4. Inflammatory cells contain phosphatases.

Comori in 1941, using his paraffin method for acid phosphatase, found that this enzyme was diminished in acute inflammations but quite often increased in chronically inflamed areas and granulation tissue, while necrotic and caseating areas were entirely free from the enzyme.

IV. Cystic Glandular Hyperplasia of the Endometrium

All cases of hyperplasia included in this study showed the most common histological pattern of endometrial hyperplasia, the proliferative type with glands in various degrees of proliferation or cystic dilatation, the so-called cystic glandular hyperplasia. There were no histological signs of secretion.

Histochemical Pattern:

There was increase in the following substances:

Alkaline phosphatase

Ribonucleoprotein

Non-specific esterase

Mucin

Stromal fat.

In the glands fat could hardly be seen. Glycogen and acid phosphatase were scanty.

Thus the histochemical pattern of cystic glandular hyperplasia represents an exaggeration of what is seen in the proliferative phase of the normal endometrium.

Similar results were obtained by Hall (1950) and McKay (1956) in extended histochemical studies.

A comment on each of these substances in cystic glandular hyperplasia will be given.

Alkaline Phosphatase:

Separate studies of lakaline phosphatase as those by Atkinson and Gusberg in 1948 and by Jones in 1952 have also assured the increase of this enzyme in cases of endometrial hyperplasia.

Ribonucleoprotein:

In another separate study of ribonucleoprotein, Atkinson in 1949 found that the increase of this substance in cystic glandular hyperplasia was patchy in the glandular epithelium, according to the degree of hyperplasia.

Glycogen

In this study, glycogen in hyperplastic endometria was found in minute amounts, like those seen in the proliferative phase of the normal menstrual cycle. Most of the previous studies of glycogen in hyperplasia gave similar results. Hughes (1945), Atkinson (1952) and McKay (1956) reported on the marked diminution of glycogen in hyperplastic endometria, whether of the cystic or adenomatous variety.

However, glycogen was found to be increased in some cases of endometrial hyperplasia (Arazac and Blanchet, 1948).

This was also noticed long ago by Binder and Neurath in 1929 (quoted by previous author). In his series, Atkinson (1952) came across one case in which hyperplasia was associated with a secretory endometrium, where glycogen was present in ample amounts in the normal but not in the cystic glands.

It is known that some cases of functional bleeding might show a secretory endometrium as a result of a corpus luteum cyst or a luteinised theca cell tumour (Te Linde, 1953). The most common finding, however, is the presence of a persistently proliferative endometrium without signs of secretion. These are the classical cases of Metropathia Haemorrhagica, in which the pathological studies of Meyer, Shroeder, and Novak and Martzloff have revealed absence of

corpora lutea from the ovaries.

Fat:

The increased stromal fat in cases of endometrial hyperplasia seems to be the result of over-stimulation with oestrogen, while the diminution of fat in the epithelium and lumena of glands points to the lack of progestational control.

Mucin:

Mucin is seen in large amounts in the lumena of the cystic glands in endometrial hyperplasia. This means that the secretion of mucin is influenced by oestrogen and not by progesterone. In the normal menstrual cycle, mucous secretion was noticed to precede the secretion of glycogen which is generally considered to be secreted under the effect of progesterone.

Acid Phosphatase:

This enzyme is markedly diminished in cases of cystic glandular hyperplasia.

This can be attributed to lack of progestational influence on the endometrium in such cases.

Non-specific Esterase:

The increase of non-specific esterase in endometrial hyperplasia confirms the assumption that in such cases there is oestrogenic overactivity.

From the previous observations and discussions of the histochemical pattern of cystic glandular hyperplasia, it appears that this condition should be accompanied by increased oestrogens.

This has been confirmed recently by Brown, Kellar and Matthew (April, 1959) in their study of the urinary oestrogen excretion in certain gynaecological disorders.

In all of the fifteen cases of cystic glandular hyperplasia included in their study, the figures for urinary oestrogen excretion were remarkably high.

ENDOMETRIAL CARCINOMA

We have seen that in carcinoma of the endometrium there is abnormal increase in the following substances:

Ribonucleoprotein

Acid phosphatase

Non-specific esterase

Lipoids.

Alkaline phosphatase is markedly diminished and in most instances absent from the cancerous growth, except in the endothelial lining of blood vessels.

Glycogen is variable.

Mucin is present in the glands in a patchy distribution.

Few histochemical studies have been carried out on carcinoma of the endometrium.

Fat was not studied in cancer of the endometrium, at least in the range of literature which I have reviewed, although this substance has received some attention in studies of the normal endometrium.

In the light of the observations in this study, as well as those of other workers, each substance will be commented on separately.

Alkaline Phosphatase

Alkaline phosphatase was the only substance which was constantly and markedly diminished in the glands of endometrial cancer. This enzyme was studied in endometrial

carcinoma by Atkinson and Gusberg (1948), Hall (1950), and by McKay and associates (1956). All of them confirmed the marked diminution of the enzyme in endometrial cancer, and its persistence in the endothelium of blood vessels.

Atkinson and Gusberg made correlation between the amount of alkaline phosphatase and the degree of differentiation of cancer. In the undifferentiated type, they found the reaction totally absent from tumour cells but present only in the endothelium of the blood vessels. In the well differentiated tumours, some reaction was seen in the cancerous cells in a patchy distribution and also in the blood vessels in their endothelium.

Jones and associates (1952) gave the results of biochemical assay of alkaline phosphatase in only three cases of endometrial cancer. The figures they obtained did not exceed what could be found in a normal endometrium. They did not display the histochemical distribution of the enzyme in those cases of endometrial cancer. Such values obtained by chemical estimation could be most probably due to alkaline phosphatase of the blood vessels.

Ribonucleoprotein

The presence of huge amounts of ribonucleoprotein is a constant finding in endometrial carcinoma. This conforms to the previous results of Stowel (1947) and Atkinson (1949). McKay (1956) found increased ribonucleoprotein in

cancerous endometrium, but in two cases which he considered adenocarcinoma in situ, there was a scanty amount of ribonucleoprotein.

Gross and Danziger (1957) found increased amounts of ribonucleoprotein in "cervical" cancer, as well as in benign proliferative conditions of the cervix.

It can be noticed that although ribonucleoprotein is increased in cancer endometrium, alkaline phosphatase is diminished. This means either that the relation of alkaline phosphatase to protein synthesis is not absolute, or that there is no further synthesis of adult proteins in cancerous cells.

Acid Phosphatase

This enzyme is one of the substances increased in cancer. In 1941, Gomori, using his old paraffin method for acid phosphatase, studied this enzyme in a variety of malignant tumours in different tissues. He found an increase in acid phosphatase in most cases of carcinoma of the prostate, carcinoma of the stomach, and in two cases out of four of carcinoma of the buccal mucosa. Three cases of cancer of the ovary, four carcinomas of the breast and four sarcomas were all negative.

Using the more recent Coupling Azo method on frozen sections, Gross and Danziger (1957) found a marked increase of acid phosphatase in cancer cervix. McKay in 1956, also using a coupling azo method on frozen sections,

reported on the increase of acid phosphatase in endometrial carcinoma.

The probability is that if those cancers in other tissues giving negative results with the old paraffin method had been studied by means of the newer techniques, acid phosphatase might have shown in greater quantities.

But why is alkaline phosphatase absent while acid phosphatase is increased in carcinoma? Could it be an ionic change which establishes the optimum medium for the action of acid but not alkaline phosphatase? And what might the mechanism of this ionic change be? These questions still await their answers.

Glycogen

Atkinson and associates in 1952 investigated glycogen in cancer endometrium by histochemical methods. In their series, they found that one third of the undifferentiated cancers and one half of the differentiated contained glycogen in a considerable quantity, comparable to that present in normal secretory endometrium. In the remaining neoplasms of the series, they found glycogen but in a scanty amount, like that in normal proliferative and in hyperplastic endometrium. They also found that the deposition of glycogen was not uniform throughout a particular tumour, but varied considerably. Ultimately they were unable to correlate the degree of differentiation of

individual cells or groups of cells to the amount of glycogen they contained.

McKay in 1956 found that the glycogen content of tumour cells in endometrial cancer was extremely variable, some tumours were devoid of glycogen, while others contained large quantities, and others still showed only patches of glycogen.

In squamous cell carcinoma of the cervix, Gross and Danziger in 1957 reported on the absence of glycogen from undifferentiated cancer cells, and its presence when cancer tissue was fully differentiated, thus behaving like the normal adult cervical squamous epithelium.

We cannot, however, compare the changes in squamous cell carcinoma of the cervix with those of endometrial adenocarcinoma, as the physiological specialities of the squamous epithelium of the cervix are quite different from those of the endometrial epithelium. Cervical glycogen does not show appreciable cyclic or menopausal changes (Wheeler and Danziger) like those seen in the endometrium.

In this study, the presence of glycogen seemed to be associated with the rapid growth of the tumour. The explanation for this observation is that the rapidly growing cancer tissues outgrow their blood supply and are relatively poorly vascularised. The relationship between poor vascularisation and glycogen deposition was discussed by Dempsey and Wislocki (1944) in studying the human placenta; and by

Wislocki and Dempsey (1945) in studying the pregnant endometrium. In such circumstances of rapid growth, anaerobic glycolysis (anaerobic glucose utilisation) prevails, as glucose whenever available is more readily utilised anaerobically than glycogen, and thus glycogen is spared. The literature pertaining to anaerobic glycolysis in rapidly growing tissues such as embryonic ones or malignant tumours, has been adequately reviewed by Reynolds in the second edition of his book "Physiology of the Uterus", in the chapter dealing with uterine respiration.

Mucin

The increase of mucin in cancer of the endometrium was not uniform. Some areas were devoid of mucin, others showed mucin in the lumena of glands or in the cytoplasm of the cancer cells. It did not follow the distribution of glycogen or any of the other substances included in this study. Thus nothing of significance could be attributed to this substance in cancer of the corpus uteri, probably different areas in the same tumour have got different potentialities.

Lipoids

In endometrial hyperplasia we have already noticed the increase in the amount of stromal fat which showed some steroid reactions. In all cases of cancer endometrium, there was an outstanding increase of lipoid, of the same

nature as that found in hyperplasia, although the amount and size of the fat globules were bigger than in the cases of hyperplasia. Fat could be seen in between the glands as well as in the glandular epithelium and in the lumena, unlike what was noticed in hyperplasia, where glands were nearly devoid of fat.

There seems to be a link between hyperplasia and cancer through this fatty substance with the steroid nature. It is in this lipoid substance that we might find a clue to the aetiology of endometrial carcinoma.

Fat is the only substance which shows a gradiant of increase from hyperplasia to cancer.

The presence of fat in the glands in cancer but not in hyperplasia means that another factor, probably of a progesterone-like effect, works in cancer.

Endometrial hyperplasia and, more precisely, the post-menopausal hyperplasia, especially the proliferative variety, is considered to be a forerunner of malignancy (Hertig, Speert, Te Linde and Novak). If this is true, there must be an underlying cause in both conditions, which at least predisposes to, though not necessarily causes, cancer. Oestrogen was always suspected to be that underlying cause, as will be discussed later. If it be oestrogen, then could this endometrial fat represent oestrogen fixed by the endometrium or elaborated there? Or perhaps

another substance with a steroid structure elaborated under the influence of oestrogen?

Non-specific esterase

This enzyme was shown to be markedly increased in cancer, thus confirming the results of McKay in his recent study of the endometrium. In carcinoma of the cervix, this enzyme was also noted to undergo significant rise (Gross and Danziger). As the reaction given is not specific to one enzyme, we cannot go too deep into the significance of this increased activity in cancer. Probably a more detailed study of this enzyme might yield more information.

To summarise the histochemical changes in cancer, we reckon that substances showing significant change are:

Alkaline phosphatase : diminishes.

Glycogen : when present is a sign of rapid growth.

Ribonucleoprotein : increases.

Acid phosphatase : increases.

Non-specific esterase : increases.

Possible Hormonal Relations of Cancer Endometrium:-

Oestrogen and progesterone have long been suspected as contributing factors to cancer. Many workers have tried to prove this relation, but no conclusive evidence was arrived at. Experiments on animals were carried out, for example those by Greene and Saxton (1938) on rabbits;

Greene and Newton (1948) also on rabbits; and by Hisaw (1950) on monkeys. (The last reference was quoted by Gillman, 1941.)

Hisaw injected castrate monkeys with various combinations of hormones for long periods of time. Oestrogen alone, progesterone alone, and oestrogen followed by progesterone, all failed to produce any abnormal growth of the endometrium. When oestrogen and progesterone were given together, however, a large polypoidal growth of the endometrium occurred. But even then, this growth was not carcinomatous.

McKay, from his "Histochemical Observations on the Human Endometrium", came to the conclusion that a progesterone-like action was suspected to take place in carcinoma of the endometrium. In two cases which he considered adenocarcinoma in situ, the histochemical pattern was similar to that of the progestational phase.

It seems that the histological picture of the normal endometrial areas when associated with adenocarcinoma can give an idea about the prevailing hormonal influence in such cases. If it is a highly proliferative endometrium, then hyperoestrinism is suspected. On the other hand, if the normal areas show the normal secretory pattern, then progesterone could be blamed.

In the recent paper of Brown, Kellar and Matthew, bits of atrophic senile endometrium were noted in conjunction

with the endometrial cancer. In such cases, however, the levels of urinary oestrogen excretion were in the normal range for menopausal women.

Oestrogen as a Predisposing Cause of Endometrial Carcinoma:

All the above considerations, however, do not rule out the role of long oestrogenic stimulation in the predisposition to cancer. Such a role has been evidenced by wide and comprehensive observations, which cannot be ignored until beaten by surer evidence.

There is a relatively higher incidence of endometrial cancer in association with conditions of increased oestrogen production. Endometrial carcinoma was found to exist in 20% of patients with feminising ovarian tumours (Novak and Novak, 1958). Ovarian stromal hyperplasia is two and a half times commoner in association with endometrial cancer (Woll, 1948; and Novak and Mohler, 1953).

Corscaden and Gusberg (1947) noted the occurrence of endometrial cancer in five patients after long continued oestrogen administration.

Cervical cancer has been produced in mice by oestrogen as reported by Garden and Li (quoted by Novak and Novak, 1958).

Novak and Novak (1958) believe that the production of aseptic pyometra in experimental animals is a factor which can prevent the appearance of cancer endometrium after

prolonged oestrogen administration.

Whatever the cause of cancer might be, the histochemical pattern of adenocarcinoma of the uterine body is a unique one, and can be helpful in at least the diagnosis and assessment of the rate of growth of the tumour.

The histochemical changes in cancer of the cervix, as Gross and Danziger emphasised, are not specific, and could be seen in some benign proliferating conditions of the cervix. In the endometrium, we have seen that cancer could be differentiated from benign hyperplasia and some other pathological conditions.

A Hint on Irradiated Cancer:-

The case of adenocarcinoma of the uterine body which had radium for one week before the operation showed large amounts of alkaline phosphatase in the cancerous epithelium, as well as in the blood vessel endothelium. There was also increased mucin secretion. The presence of alkaline phosphatase in cancer after irradiation may be regarded as a reversion of the cancer process back to the normal proliferating mechanism. However, it might be no more than a reaction similar to that seen in inflammatory conditions, where alkaline phosphatase is seen in large amounts, especially as it is associated with increased mucin production, and this also occurs in inflammatory conditions.

SUMMARY

Seven substances were studied by histochemical methods in the human endometrium, at different phases of the normal cycle, and in certain pathological conditions.

The histochemical pattern in each condition was presented and the significance of these observations was discussed.

Of the seven substances, endometrial fat and non-specific esterase need further detailed separate studies.

PART II

THE HUMAN PLACENTA

OBSERVATIONS AND DISCUSSION

O B S E R V A T I O N S

Observations on the human placenta will be dealt with under the following headings:

A. The Normal Placenta

I. The placenta of the first trimester.

II. The placenta of the second trimester.

III. The placenta of the third trimester.

IV. The placenta at term.

B. The Pathological Placenta

I. The placenta in post-maturity.

II. The placenta in pre-eclamptic and eclamptic toxaemia.

III. The placenta in essential hypertension in pregnancy.

IV. The placenta in renal failure in pregnancy.

V. The placenta in diabetes mellitus in pregnancy.

VI. The Vesicular Mole.

A. The Normal Placenta

I. The First Trimester

Eight placentae in the early period of pregnancy were obtained from surgical termination carried out for cardiac or mental conditions. The ages of these placentae were as follows:

1 placenta of 7 weeks gestation (therapeutic evacuation)

2 placentae of 8 weeks gestation (Hysterectomy)

1 placenta of 9 weeks gestation (Hysterectomy)

2 placentae of 12 weeks gestation (Hysterectomy)

2 placentae of 13 weeks gestation (Hysterectomy)

Notes on Histology:

1. In all the above-mentioned placentae, the cellular as well as the syncytial trophoblast were present.
2. The foetal blood vessels could be seen as early as 8 weeks gestation.
3. The main chorionic villi were large with a thin stroma and small fibroblastic cells.

Histochemical Observations:

Alkaline Phosphatase:

At 7, 8 and 9 weeks - The alkaline phosphatase was present in the young chorionic villi burrowing in or adjacent to the decidua, its site being in the covering syncytial trophoblast. In the slide from a 9 weeks placenta the reaction could be seen in developing villi while still attached to the precursor cytotrophoblast; the site of the reaction is also the syncytium.

At 8 and 9 weeks a colour reaction was noticed in the stroma of some chorionic villi as a thread just beneath the trophoblastic cover, but in these sites the reaction was

also present in the control slides and in the slides treated with Von Kossa for detection of calcium. Therefore, this was just calcium reacting with the phosphate of the buffered solution.

At 12 and 13 weeks - the alkaline phosphatase started to show in parts of the trophoblastic covering of the main (secondary) chorionic villi. The enzyme was also present in the whole covering of the small tertiary villi. Fig. 53.

The reaction was identical in the paraffin Gomori sections as well as in the frozen Azo method.

Acid Phosphatase:

7-9 weeks - acid phosphatase was found in the cytotrophoblast in the cell masses, but not in Langhans' layer.

In the syncytium, at 7 weeks, acid phosphatase was only seen in the villi burrowing into the decidua. At 9 weeks, the syncytium of the main villi, starts to show a reaction. Fig. 51.

In the decidua, acid phosphatase was seen in decidual cells and endometrial glands.

At 12 and 13 weeks - by the frozen Azo method there was a universal colour reaction of the enzyme in the syncytial covering of the chorionic villi and in the differentiating trophoblastic cell columns. In the decidua a faint reaction was present in decidual cells. Fig. 52.

By the paraffin method no reaction was revealed.

Non-specific Esterase:

This enzyme was only studied in the 12 and 13 weeks placentae. By the Frozen Azo method, the reaction was strongly positive in the trophoblastic elements and in some scattered decidual cells. Fig. 54.

By the paraffin method the reaction was not revealed.

Glycogen:

At 7 weeks - only decidual tissue was found in the section and there was plenty of glycogen in the glands, stroma cells and surface epithelium.

At 8 weeks - glycogen was seen in the cellular trophoblast and in the stroma of the villi. It was quite noticeable in the walls of the thick foetal blood vessels.

At 13 weeks (Fig. 59) - glycogen was present in moderate amounts in the differentiating cell columns and in the layers of the cellular trophoblast. It was also present in the stroma of the villi, though in smaller amounts.

Mucin:

In these young placentae mucin was only seen in the decidual glands, in their lumena and in areas of the cell columns where chorionic villi are developing.

Calcium:

Black deposits were seen in the small tertiary villi. In the main villi it was seen as fine deposits in the stroma

at 9 weeks, and at 13 weeks these fine deposits were only seen beneath the trophoblastic covering of the villi.

Ribonucleoprotein: (Fig. 61)

Cytoplasmic basophilia is marked in the syncytium of all the chorionic villi. It is noticed to a less extent in Langhans layer. Cytotrophoblastic cell columns and cell islands show a marked degree of basophilia.

Lipoids:

Sudan IV - In the decidua fat was present in many decidual cells, in the surface epithelium and in decidual glands. Fig. 57.

In the chorionic villi - the syncytium was very rich in fat which was noticed as heaps of big fat globules easily seen by the low power of the microscope. Fig. 55. (12 weeks placenta.)

Schultz test - no blue colour was seen in the specimens subjected to this test.

Birefringence - no birefringence was noticed in the foetal parts of these young placentae, but in a case of 4 weeks abortion, fat in the decidua was doubly refractile. Fig. 58.

II. The Placenta of the Second Trimester

Four placentae of the second trimester were available. Three were obtained from hysterectomy specimens (pregnancy terminated for pulmonary tuberculosis and mental disease).

One placenta at six months (from an unexplained miscarriage) was considered normal as it showed no pathological histology. The dating of the four placentae was as follows:

1 placenta at 16 weeks gestation

1 placenta at 18 weeks gestation

1 placenta at 20 weeks gestation

1 placenta at 25 weeks gestation.

Notes on Histology:

1. At 20 weeks, very little villi showed Langhans cells under the syncytium.
2. The size of the main (secondary) chorionic villi is still remarkably large at this age of the placenta.

Histochemical Observations:

Alkaline Phosphatase:

At 16 weeks, alkaline phosphatase was present in parts of the syncytium. The Langhans layer was negative.

At 20 weeks, (Fig. 62) all the stem (primary) villi showed a strongly positive reaction in the syncytium, while the syncytium of the secondary and tertiary villi was partially positive. The villi in direct contact with the decidua showed colour reaction in the whole syncytium.

At 25 weeks, the reaction was present in the syncytium of all the villi.

The distribution of the enzyme was the same in paraffin and in frozen sections while the intensity varied.

The decidua was always negative except for the lining of blood vessels when seen in the sections.

Acid Phosphatase:

Frozen Aso Method (Fig. 63, 20 weeks placenta). The reaction was present in all the syncytium homogeneously, its intensity increasing with increased dating of pregnancy. The decidua was negative except for a faint reaction in some decidual cells.

By the Paraffin Method of Gomori, the reaction was patchy and mostly nuclear in the syncytium and stroma groups of villi.

Non-specific Esterase: (Fig. 64)

At 20 weeks (Fig. 64), a reaction was present in the cytoplasm of the covering syncytium of all the villi. Many secondary and tertiary villi showed a reaction in the stroma, while the stroma of the stem villi was constantly negative. In the decidua, there was a reaction in the epithelium of some glands, in many cells of the decidua and in the lining or within the lumena of some blood vessels.

Glycogen: (Fig. 68, 20 weeks placenta, Best's)

By both stains (Best's and Chromic Acid Schiff), glycogen could always be seen in:

1. Decidual cells.
2. Stroma of stem villi in Wharton's jelly.
3. Walls of thick foetal blood vessels.

Mucin:

The only mucicarmine stainable material in the placenta was the hyaline diffuse matrix in the trophoblastic cell columns and cell islands.

Calcium: (Fig. 69,18 weeks placenta)

Fine black deposits were occasionally seen in the stroma of a villus. The decidua showed no such black deposits.

Ribonucleoprotein:

Cytoplasmic basophilia is moderate in the syncytium. The cytotrophoblast represented in this period of pregnancy by the cell columns and cell islands still shows marked basophilia in the cytoplasm of its constituent cells which are present in the hyaline matrix. The wandering trophoblastic cells in the decidua also possess this basophilia in their cytoplasm.

Lipoids: (Fig. 65, 20 weeks placenta)

Sudan IV - Low Power: Fat globules were seen in the sites where non-specific esterase was found, namely in the stroma of the chorionic villi with non-specific

esterase activity and in the decidua in some decidual cells, glands and blood vessels.

High Power: Minute fat droplets could be seen in the cyncytium of many of the chorionic villi.

Schultz Test: (Fig. 66, 20 weeks placenta)

This test was positive in the stroma of the chorionic villi which showed fat and non-specific esterase activity. The colour was also seen inside a blood vessel in the decidua (probably cholesterol of the maternal blood).

Birefringence, was noticed within the decidual blood vessel which gave a colour reaction with Schultz test.

III. The Placenta of the Third Trimester

Six placentae from pregnancies which ended prematurely but had no obvious clinical cause or pathological histology have been taken as normal. Their ages were between 28 and 35 weeks of pregnancy.

Notes on Histology:

1. At 28 weeks many new villi were seen developing from the trophoblastic cell columns, Fig. 70.
2. At 30 weeks the chorionic villi showed marked variability in their size and texture; some are large with fine, loose (ageing) stroma, while others are small with a denser and a more cellular stroma, Fig. 72.

3. At 34 weeks Tenney's nodes were remarkable, Fig. 73.

4. At 35 weeks, while criteria of ageing are noticed, new villi are still developing from the cytotrophoblast, Fig. 71.

Histochemical Observations:

Alkaline Phosphatase: (Fig. 74, 28 weeks placenta)

The reaction was universally present in the syncytium of the chorionic villi in sections treated by the Frozen Azo and the paraffin Gomori methods.

Acid Phosphatase:

Frozen Aso Method (Fig. 75). The reaction was present in the syncytium of all the chorionic villi and in the decidual cells. The reaction was also seen in the necrotising cells in the fibrinous layer at the margin of the decidua.

In the paraffin sections incubated at pH 5.2 observations were identical, while with the lower pH 4.8 only a nuclear reaction could be seen.

Non-specific Esterase: (Fig. 76, 28 weeks placenta)

This enzyme was only present in a few decidual cells.

Glycogen: (Fig. 77, 35 weeks placenta, Best's)

By both stains glycogen was constantly shown in the decidual cells, though in less amounts than it was seen in earlier pregnancies. In chorionic villi it was more

abundant in the stroma of the stem villi than in the stroma of the secondary villi. The walls of the blood vessels also contain glycogen.

Calcium:

Blackish argyrophyl deposits were occasionally seen in the stroma of a villus and in one case (35 weeks) as several deposits in between the chorionic villi.

Ribonucleoprotein:

Cytoplasmic basophilia in the syncytium was less than noticed in the first two trimersters of pregnancy.

Lipoids:

Sudan IV: Minute sudanophilic fat droplets are seen in some decidual cells. Thus the decidual fat is getting less and less as pregnancy advances. In a very few instances fat could be seen in the syncytium.

Schultz Test and Birefringence were negative in the specimens examined.

IV. The Placenta at Term (39-41 weeks)

Fifteen normal placentae at term were submitted for histochemical investigation. In the earliest six placentae the enzymatic investigation by the frozen method was not yet adopted.

Notes on Histology:

The full picture of normal ageing of the placenta was obvious in all the cases examined, such as:

1. Fibrin nodes as small areas of hyalinisation scattered in the field of healthy placental villi.
2. The fibrin layer of Neutabuch as a thin irregular layer of hyalinisation at the border between the placental villi and the decidua.
3. Tenney's nodes: clumping of the nuclei of the syncytium in aggregations and stippling of their chromatin is marked.
4. The main (secondary) villi are of more or less the same size and texture with a thin syncytium all over.
5. No more developing villi showed themselves in the sections.

Histochemical Findings:

Alkaline Phosphatase:

By the Azo Frozen Method (Fig. 79) the reaction was uniformly present in the syncytium of all the chorionic villi in the cytoplasm. The deposits were heavier than seen at any age of immaturity. The stroma of the villi was negative. The decidual structures were also negative.

By the paraffin method of Gomori, the reaction was usually exaggerated, with the addition of a nuclear reaction sometimes.

Acid Phosphatase:

Frozen Azo Method (Fig. 80): The deposition of the pigment was marked in the syncytium of all the chorionic villi while the stroma of the villi was negative.

The decidual structures were negative except in a few instances where there was a reaction in some decidual cells and in the necrotising cells in the layer of Neutabuch.

Paraffin Method of Gomori (Fig. 81): At the higher pH 5.2 the reaction took the same distribution as in frozen sections, being located in the cytoplasm (high power) of the syncytium but sometimes both cytoplasm and nuclei were stained. At the lower pH 4.8 the reaction was only nuclear in most of the cases. The decidua sometimes showed a reaction in the decidual cells.

Non-specific Esterase: (Fig. 84 Frozen Azo)

This enzyme was only found in some decidual cells, and in the thin paraffin sections the reaction was marked in lymphocytes.

Glycogen: (Fig. 82), Best's)

Glycogen was present in decidual cells in small amounts. It could still be seen in the stem villi (in Wharton's jelly) and in the walls of foetal blood vessels. The main chorionic villi are negative for glycogen.

Calcium:

Small calcium deposits could be detected with the naked eye. Using the microscope, these prove to be flakes of calcium in between the villi, especially near the maternal surface of the placenta.

Ribonucleoprotein: (Fig. 83)

Cytoplasmic basophilia in the syncytium was less than was noticed in the third trimester placenta.

Lipoids:

Sudan IV (Fig. 85):

In the decidua, fat droplets were seen near the margin. Fat was also encountered in some villi which apparently were degenerating; the same was noticed in the remains of trophoblastic elements. Fat in the syncytium could not be easily seen in the fifteen micron thick frozen sections counterstained with haematoxylin, although sometimes dust-like granules could be seen on focussing up and down on the fairly thick sections.

Schultz Test (Fig. 87):

The characteristic colour was seen in the decidua in one case.

Birefringence:

The layer of fat at the margin of the decidua showed birefringence which was more marked in thicker sections. In sections of the same thickness as those stained with Sudan IV, fat droplets were patchy and did not show a definite layer as in the thick sections.

Digitonin Test for the Identification of Fat in the Placenta:

Treating the frozen sections in all the cases examined for several hours with 0.5% digitonin solution in 50% alcohol, then repeating the mentioned three tests for fat, there was no change in the amount or character of fat described in the placenta.

We conclude from this finding that there is no free cholesterol in the placenta, foetal or maternal, in normal pregnancy.

B. The Pathological Placenta

I. The Placenta in Post-maturity

Eight postmature placentae were submitted for histochemical examination. Their ages were more than 42 weeks of gestation.

Notes on Histology:

1. The outstanding naked-eye picture is the extensive calcification. Some of the placentae had a fragile maternal surface and could be said to simulate a crusty loaf of bread.
2. The haematoxylin and eosin-stained sections (Fig. 88) showed extensive hyalinisation in the chorionic villi and adjacent decidual margin.

Histochemical Observations:

Alkaline Phosphatase:

Azo Frozen Method: (Fig. 90). The black deposits of the azo compound were so heavy that the section could be distinguished from that of a normal placenta at term by the naked-eye appearance. Microscopically, these depositswere located in the syncytium of all the villi while the stroma of the villi was completely negative. The decidua was also negative.

Paraffin Method of Gomori: The distribution of the enzyme activity was the same as in the frozen method with the addition of a faint nuclear reaction in the stroma of the villi.

Acid Phosphatase:

Frozen Azo Method: (Fig. 91). The reaction was also stronger than in the normal placenta at term. The site was the syncytium of all the villi, apparently in the cytoplasm though the reaction is too heavy for sharp demarcation to be precise. There was also a patchy reaction in groups of decidual cells.

Paraffin Method of Gomori: The reaction is also very heavy, its site is the cytoplasm of the syncytium at the outer border. A milder and patchy reaction occurred in some decidual cells. This was noticed at both pHs. In two cases, however, the reaction was only nuclear at the lower pH 4.8 in nuclei of syncytium and stroma.

The black colour was seen in the calcium deposits but that colour persisted in the control slides.

Non-specific Esterase:

In the frozen azo slides (Fig. 92) traces of colour reaction could be seen in the syncytium of the villi in some cases. The stroma of the villi was completely negative. The decidua showed deposits of the Azo compound in decidual cells, glands and in the lining of decidual blood vessels.

In the paraffin azo slides the reaction was only in decidual cells.

Glycogen:

Glycogen was present in the decidual cells, in the stroma of stem villi and in the walls of thick foetal blood vessels. The amount as a whole was less than in the normal placenta at term.

Calcium: (Fig. 89)

Apart from the calcium seen by the naked eye, microscopic examination of the sections stained by the Von Kossa technique revealed many groups of calcium flakes, more abundant than in the normal placenta at term. The site of these calcium deposits is between the villi, especially near the maternal surface. The fine stromal black reaction with Von Kossa was not seen.

Ribonucleoprotein:

Cytoplasmic basophilia in the syncytium was very faint.

Lipoids: (Fig. 93)

Sudanophilic fat was seen in the stroma of degenerating villi. The completely hyalinised areas were negative for fat. The decidua contained very scanty fat.

II. The Placenta in Pre-eclampsia and Eclampsia

The number of cases submitted for histochemical examination was eighteen:

9 at term

4 at the 38th week of pregnancy

5 between the 30th and the 37th weeks of pregnancy.

Note on Histology:

Histological criteria of ageing were quite remarkable even in the youngest placenta of this group (Fig. 94, 30 weeks eclampsia). Infarctions were quite a common finding. Intimal proliferation and thickening of the media of the spiral arderioles were sometimes seen.

Histochemical Observations:

Alkaline Phosphatase: (Fig. 96)

The colour deposits are so heavy that a 30 weeks placenta in pre-eclampsia may give a reaction similar to or even more than a normal placenta at term. The site of enzymic activity is the cytoplasm of the syncytium and the

necrotic cells in the decidual margin.

Acid Phosphatase: (Fig. 97)

This enzyme was also increased but not to the extent of alkaline phosphatase.

Non-specific Esterase: (Fig. 98)

This enzyme which is supposed to disappear almost completely from the syncytium in the later period of pregnancy is present in appreciable amounts. The reaction in decidual cells is also heavier than normal.

Glycogen:

Glycogen seemed to diminish in decidual cells and increase in the stroma of the stem villi.

Calcium:

Deposits were more frequent at a younger age than normal.

Ribonucleoprotein:

Cytoplasmic basophilia was markedly less than in a normal placenta of the same age. (Compare Fig. 99, 30 weeks eclamptic placenta, with Fig. 100, 30 weeks normal placenta.)

Lipoids:

Sudan IV - The degenerative fat in the foetal and decidual parts of the placenta was markedly increased; that is, the fat in the necrotising villi and decidual cells. The layer of fat which was noticed in the normal

placenta at term in the decidua was heavier in pre-eclamptic placentae (Fig. 102). Whenever an infarction happened to show in the section excessive fat was seen in the infarcted tissue, especially at the periphery (Fig. 106), while the centre of the infarction showed variable amounts of fat according to the degree of degeneration.

In the decidua fat was also noticed in the walls of decidual blood vessels which are actually thick (Fig. 104). In the healthy-looking chorionic villi, fat was seen in the stem villi beneath the syncytium and in the secondary villi adjacent to them.

Schultz Test:

This test was positive in one case giving the characteristic blue-green colour in one area of the decidua.

Polarised Light:

The only fat which showed birefringence in the pre-eclamptic placentae was that in the decidua (Fig. 101).

III. The Placenta in Essential Hypertension

Six placentae from pregnant women with essential hypertension were examined; three of them were at term and three were of 36, 37 and 38 weeks gestation.

Notes on Histology:

There was a remarkable thickening of the blood vessels in the decidua and stem villi. The fibrous stroma of the

of the chorionic villi looked dense and hyaline in parts instead of being fine and yielding to the widely open vessels in the normally ageing chorionic villi. Syncytial atrophy was not marked as in placentae of toxaemia. The intervillous blood spaces did not show fibrin deposits and looked patent as in normal placentae.

Notes on Histochemical Changes:

Unlike the placenta in pre-eclamptic toxaemia, there was no increase in the enzymes. Alkaline phosphatase, acid phosphatase and non-specific esterase had the same amount and distribution as in normal placentae of the same period of gestation.

Fat and glycogen, as well, showed no deviation from normal.

Calcium deposits were less frequent than in toxaemic placentae.

IV. The Placenta in Renal Failure

One placenta (36 weeks) was obtained from a case of chronic nephritis and another from a typical case of type II nephritis at 37 weeks pregnancy. Both cases showed impaired renal function.

Histochemical Pattern:

Compared to placentae of normal pregnancies of corresponding ages, these two placentae showed the following characters:

1. Increase in alkaline phosphatase.
2. Frequency of calcium deposits.
3. Some increase of fat in the decidua (Fig. 107).

V. The Placenta in Diabetes Mellitus

Ten placentae were available from pregnant women suffering from Diabetes Mellitus. Pregnancy in those patients was terminated at periods between 34 and 36 weeks of gestation. One placenta was obtained by hysterotomy from a severely diabetic patient with a raised blood pressure at the twelfth week of gestation.

Histochemical Observations:

1. Enzymes were increased more than in normal placentae of the same age, but not to the extent of their increase in toxaemia.
2. Glycogen was not increased; on the contrary, it seemed to be less than in a normal placenta of the same age.
3. Fat in the decidua was more than normal while some degenerated villi showed fatty infiltration.
4. Calcium deposits were frequent.
5. Cytoplasmic basophilia was not less than in normal placenta at a similar date of gestation (Figs. 108 and 109).

In the 12 weeks diabetic placenta, two marked differences from the normal 12 week placenta were noticed:

1. Alkaline phosphatase was present in all the syncytium covering the chorionic villi, while in the normal placenta it was present only in parts of the syncytium at 12 weeks gestation.
2. The syncytial fat, which was considered by Wislocki and Bennet to represent the steroid hormones of the placenta, was not as abundant as in a normal 12 weeks' placenta (Fig. 110).

VI. The Hydatidiform Mole

Two vesicular moles were available in the early period of this study. Enzymic investigation by the frozen azo methods was not yet adopted. The period of amenorrhoea in one case was 10 weeks, and in the other was 13 weeks.

Notes on the General Histology:

1. Both moles exhibited the characteristic vesicular transformation of the chorionic villi.
2. The covering trophoblast was thin and attenuated in some parts, while in other parts it was active and heaped in many layers.
3. In one of the two moles, the trophoblast was rather active and burrowing into the decidua, which showed many blood vessels of a moderate size.

Histochemical Observations:

Alkaline Phosphatase: (Fig. 113, Paraffin Method)

This enzyme was present in the 13 weeks specimen: in the active trophoblastic cells, in some parts of the syncytial covering of the villi, and in the trophoblast invading the decidua.

The site was the cellular membrane, the nuclear membrane and the Nucleolus.

Acid Phosphatase: (Fig. 114, Paraffin Method)

There was only a nuclear reaction in some cells in the decidua.

Glycogen:

Glycogen was present in the stroma of chorionic villi and in the proliferating trophoblastic cells. The amount was definitely more than in normal placentae of the same age.

The hyalinised acellular areas of the stroma and the trophoblast contained no glycogen.

In the decidua the amount and distribution of glycogen was the same as in a normal pregnancy of the first trimester.

Mucin:

The mucicarmine stain was positive in parts of the stroma of the chorionic villi and within the cytoplasm of the cellular trophoblast.

Ribonucleoprotein: (Fig. 115)

Cytoplasmic basophilia was marked in the active trophoblast, both cellular and syncytial.

Calcium:

The Von Kossa reaction was completely negative contrary to what was seen in the normal villi of early pregnancy.

Lipoids:

Sudan IV -

A. In the decidua, fat was present in enormous amounts.

B. In the syncytium, heaps of fat granules were present in the syncytium covering actively proliferating cellular masses. The amount of fat was less in the syncytium covering the vesicularly transformed villi, while in the completely hyalinised syncytium there was no fat.

C. In the stroma of some chorionic villi, finely dispersed fat granules were seen.

Schultz Test:

This test was positive only in the sites of stromal fat, which proved after the Digitonin test to be free cholesterol.

Birefringence:

Fat in the decidua was doubly refractile (Fig. 116).

Stromal fat which gave a positive Schultz test showed the doubly refractile needles of free cholesterol.

Note on the use of Digitonin in Identification of Fat in the Placenta in Abnormal Pregnancies

Incubation of frozen sections in 0.5% digitonin in 50% alcohol, then repeating the three fat tests, revealed no change in the amount or character of fat present in all the pathological placentae examined, except in hydatidiform moles.

This leads us to conclude that there is no free cholesterol in the placental tissues in the pathological conditions studied here except in the degenerating villi of hydatidiform moles.

D I S C U S S I O N

THE NORMAL HUMAN PLACENTA

Alkaline Phosphatase

This enzyme starts to show activity in the syncytium of the main chorionic villi at about the third month of gestation. In placentae younger than three months, the syncytium shows alkaline phosphatase in only three sites:

1. the villi which are in close association with the decidua;
2. the villi which are just developing and still attached to the mother cytotrophoblast; and
3. the small terminal tertiary villi.

After three months, alkaline phosphatase in the syncytium of all placental villi increases gradually and reaches a maximum at term.

The cellular trophoblast shows no trace of the enzyme.

In the decidua, alkaline phosphatase is only seen in the lining of blood vessels during the first half of gestation.

Calcium deposits in the junctional zone of the decidua near term gave a reaction in the paraffin Gomori method, but this reaction was absent in the control slides. With the frozen Azo method calcium deposits in the same

sites took the pinkish colour of Grenacher's alum carmine counterstain, but they did not give the coupling reaction characteristic of the enzyme.

Acid Phosphatase

In this study, acid phosphatase was seen in the syncytium of the main chorionic villi as early as the ninth week of gestation, and by the thirteenth week almost the whole syncytium showed the reaction for acid phosphatase. This reaction gradually increased as term was approached. Before the ninth week, acid phosphatase in the syncytium showed the same limited distribution as that of alkaline phosphatase.

Unlike alkaline phosphatase, acid phosphatase was seen in the cellular trophoblast, in cell masses and in some parts of the Langhans layer.

In the decidua, a reaction for acid phosphatase was seen in decidual cells, but as pregnancy advanced the reaction became less evident. In the first two months of pregnancy, the enzyme could be seen in the glands of the pregnant endometrium.

The frozen azo acid phosphatase reaction, as was the case with alkaline phosphatase, was seen in the cyotplasmic part of the cell.

By the paraffin method of Gomori, acid phosphatase reaction was mostly nuclear, the same as reported by

Demspey and Wislocki (1947), and by Wislocki and Dempsey (1946 and 1948). The nuclear reaction in most instances was noticed in the same cell which gave only a cytoplasmic reaction with the coupling method.

In this study, the distribution of acid phosphatase in the placenta was somewhat different from that described by Wislocki and Dempsey in their study using only the old paraffin Gomori method with lead nitrate as substrate. The differences are:

(a) The earliest age at which acid phosphatase could be seen by Wislocki and Dempsey in the syncytium was four months of gestation, while here it has been noticed in the syncytium of the main chorionic villi at nine weeks, and at an earlier date in the syncytium of the villi attached to the decidua or to the mother cytotrophoblast and in the syncytium of the tertiary villi.

(b) In 1947, Dempsey and Wislocki described a reaction which they ascribed to acid phosphatase, in the stroma of chorionic villi of young placentae. Such reaction I have found in the paraffin slides treated with lead nitrate, but not with the azo coupling method. The very same reaction was seen in the slides treated with Von Kossa for

the detection of calcium. This stromal reaction, therefore, does not represent an enzymic activity but shows the presence of inorganic calcium phosphate.

Significance of Phosphatases in the Placenta

Early in pregnancy, the growing embryo depends for its nutrition on the decidual stores. The presence of phosphatases in the syncytium of villi which are burrowing into the decidua makes one think of a related function. The means by which the stored nutrition in the decidua reaches the embryo is not yet known. The role of phosphatases in transport in the kidneys and intestines is well known. One might therefore assume a similar role in the transport of nutrition from the decidua to the young embryo. The presence of phosphatases in the terminal tertiary villi could also be attributed to an absorptive function, since those villi are generously provided with maternal blood from which they can select the nutritional ingredients necessary for the foetus.

By the third month, the nutritional stores in the decidua are nearly depleted, and the foetus has developed its own stores of nutrition, mainly in the liver. By that time the placenta is well formed, and the main bulk of villi can gain access to the maternal blood pool within the placenta.

The syncytium of the main villi, therefore, is the seat of many physiological processes. One of its main functions is the transfer of oxygen and metabolites. On the other hand, it is the probable seat of synthesis and secretion of specialised sunstances such as the placental hormones.

As the foetus and the placenta grow, there is more need for such physiological processes in which phosphatases are implicated.

The presence of acid phosphatase in decidual cells abundantly in the first few months of gestation can be explained by the fact that the stored substances in the decidua, glycogen, fat and protein, have to be broken down to simpler constituents to make them absorbable by the neighbouring villi in the decidua.

Acid phosphatase in the cellular trophoblast seems to be concerned with trophoblastic growth, since this enzyme has always been noticed in growing tissues, whether the growth is normal or pathological such as cancer.

The presence of alkaline phosphatase in the endothelium of blood vessels of the decidua in the first half of pregnancy may be related to the necessity of transfer of metabolites from the maternal blood to be stored in the decidua to answer the needs of the foetus until the placenta is well established.

Ribonucleoprotein

Ribonucleoprotein was recognised as cytoplasmic basophilia in the cellular and syncytial elements of the trophoblast. In the trophoblastic cover of chorionic villi, cytoplasmic basophilia was more intense in the syncytium than in the Langhans layer. In the syncytium it was noticed to diminish as term approached.

Possible Role of Ribonucleoprotein in the Placenta

1. Role in Placental Growth:

Ribonucleoprotein preponderates in young and actively growing tissues, including the trophoblast, whose growth is more marked in early pregnancy. Growth means protein synthesis, in which ribonucleoprotein is involved.

2. Role in Nutrition of the Embryo:

The cytoplasmic basophilia of some decidual cells is also marked in the first trimester placenta. This can be regarded as a protein store to be used for the nutrition of the growing embryo before the placental circulation is well established.

3. Role in Hormone Formation:

Wislocki and Dempsey (1946) tried to correlate the amount of cytoplasmic basophilia in the trophoblast with chorionic gonadotrophin levels in the blood and urine of pregnant women.

In support of such relation are:-

1. Human chorionic gonadotrophin levels in blood and urine are much higher during the first trimester of normal pregnancy than later in pregnancy (Loraine and co-workers, 1958).
2. Dempsey and Wislocki (1945 and 1946) were impressed by the analogy between the cytoplasmic basophilia of the trophoblast and that of the anterior pituitary cells which secrete pituitary gonadotrophins.

Against that assumption are:-

1. It is generally believed that chorionic gonadotrophins are elaborated by the cellular trophoblast, including Langhans cells. The syncytium which is the main site of ribonucleoprotein in the placenta was not identified in the tissue cultures producing chorionic gonadotrophins (Grey and associates).
2. Ribonucleoprotein is diminished in pre-eclamptic placentae while the levels of chorionic gonadotrophins in blood and urine of women with severe pre-eclampsia are significantly higher (Loraine and Matthew, 1950).

Relation between Phosphatases and Ribonucleoprotein in the Placenta

An inverse relation between ribonucleoprotein and phosphatases in the placenta. Ribonucleoprotein is abundant in early pregnancy and diminishes as pregnancy advances, whereas phosphatases are scanty in early pregnancy and increase as pregnancy advances. This has been correlated by Reynold to the function of the placenta being, in early pregnancy, a storage depot in which energy is expended in anaerobic processes, including protein synthesis, and in the later part of pregnancy primarily an organ of exchange, where phosphorylation plays an important role.

Glycogen

Glycogen in the placenta has been meticulously studied by Dempsey and Wislocki in various histochemical studies, (1944, 1945, 1946, and 1948). The findings in this study agree with their results on glycogen distribution. Glycogen is abundant in the cellular trophoblast, except the Langhans layer which is devoid of glycogen. The syncytium contains no glycogen. The stroma of the villi showed variable amounts of glycogen; the stem villi contained a lot of glycogen in the stroma and in the walls of the big foetal blood vessels. The more periferal the villi were, the less was the amount of glycogen within the stroma.

In the decidua, glycogen is abundant in the first trimester in decidual cells especially in the zona compacta, while decidual glands also show plenty of glycogen in the first two months of pregnancy.

Thus glycogen in the placenta, including decidua, is abundant early in pregnancy, but as pregnancy advances the amount of glycogen gradually diminishes.

Dempsey and Wislocki (1944) postulated that the significance of glycogen storage in the placenta might be to furnish energy for anaerobic respiration in cells the respiratory metabolism of which was insufficient for their needs, either because of a sluggish oxidative metabolism or a deficient circulation or both. They argued that the deposition of glycogen in liver and muscle occurred in the following way: either these organs contained special mechanisms superimposed on the general one, or they represented entirely special cases in which glycogen storage was determined largely by the relative rates of delivery and utilisation of glucose.

The diminution of glycogen in the maternal and foetal parts of the placenta after the first trimester, is considered to be due to the taking over of the function of glycogen storage by the foetal liver.

Relationship between Glycogen deposition and Phosphatases

The phosphorylation and dephosphorylation which take place before glycogen deposition necessitates the

involvement of phosphatases. This relationship was confirmed by the studies of Wislocki and Dempsey (1945) on animal placentae at various stages of gestation. Although there were certain discrepancies in the location of phosphatase in different species, the enzyme was always found somewhere between the maternal blood (containing glucose) and the site of glycogen deposition.

The fact that the syncytium is rich in phosphatases but devoid of glycogen is simply because phosphatases have got other functions in the syncytium, such as absorption of different nutrients from maternal blood, and the elaboration and secretion of some other substances into the maternal blood.

Fat

In this study, fat in the normal placenta was found in the following sites:

1. In the syncytium.
2. In the stroma of some healthy chorionic villi.
3. In the decidua.
4. In degenerating chorionic villi and decidua.

1. Syncytial Fat:

In the syncytium, fat droplets were found all through the period of gestation, being more abundant early in pregnancy and diminishing towards term.

2. Stromal Fat:

Fine granules of fat could be noticed in the stroma of some chorionic villi. This was noticed in the second trimester placenta, but as it was not present in all the chorionic villi, one cannot exclude the possibility of its presence in the stroma of the villi of younger or older placentae.

3. Decidual Fat:

In the decidua, fat is most abundant in the first trimester, but gradually diminishes as pregnancy advances.

In the first trimester, decidual fat is present in the majority of endometrial glands, in the surface epithelium, and in some decidual cells. In the decidual cells it is more abundant, and the size of lipoidal droplets much larger, in the superficial layer of decidua just beneath the surface.

In the second half of pregnancy, as Gillman noticed, fat was present in a band of decidual cells just beyond the fibrinous layer of Nitabuch. This band of lipoid containing decidual cells was noticed even at term, but could be more definitely identified in thicker sections.

Birefringence of decidual fat could sometimes be seen.

Schultz test was positive in the decidua in one case at term.

4. Degenerative Fat:

Fat was also encountered in a few degenerated chorionic villi and decidual cells at the margin of the decidua at or near term. This degenerative fat is a sign of the physiological ageing process in the normal placenta.

The Nature of Placental Fat

Syncytial Fat:

In discussing the nature of syncytial fat in the placenta, reference has to be made to the work and conclusions of Wislocki and Bennet (1943), Dempsey and Wislocki (1944), and Wislocki and Dempsey (1948). These investigators were led to the assumption that syncytial fat is related to the metabolism of placental steroid hormones, oestrogen and progesterone, since syncytial fat in their hands gave positive reactions which, taken collectively, were considered indicative of steroid hormones (Bennet, 1940; Pollock, 1942; Dempsey and Basset, 1943). These reactions are:

1. Birefringence.
2. Lipoidal soluble autoflorescence.
3. Plasmal Schiff reaction.
4. Phenyl hydrazine test.
5. Sulphuric acid reactions.
6. Indophenol oxidase (Nadi Reactions, Pearse, 1953).
7. Reduction oxidase indicator dyes (dehydrogenase).

Birefringence:

I could not find any birefringent fat in the syncytium in all placentae examined as fresh or formalin-fixed preparations. Froboese (1924), quoted by Wislocki and Bennet (1943), also could not detect doubly refractile fat in the syncytium. Birefringence of syncytial fat was reported by Grosser (1927), Wislocki and Bennet (1943), and by Dempsey and Wislocki (1944).

Autoflorescence:

This is detected by a special microscope with ultraviolet light directed from a carbon arc, then passed through a quartz condensing and collimating lens. Such a microscope was not available during the course of this study.

Plasmal Schiff Reaction:

This reaction was carried out in this study on a number of normal placentae at different ages of gestation. A homogeneous violet colour was produced in the syncytium in all specimens, but differences in the intensity of the colour were not significant. When positive, this reaction only indicates the presence of unsaturated substances.

Phenyl hydrazine test:

In our study, the test was applied to a collection of normal placentae in all trimesters and at term. The characteristic yellow colour was homogeneously produced in

the syncytium of all placentae examined, and here also differences in the intensity of colour were not significant. The phenyl hydrazine test indicates the presence of ketones and aldehydes, and as it was negative after treatment of control sections with acetone, it could be taken to indicate fat in the form of ketones or aldehydes.

Sulphuric acid reactions:

Concentrated sulphuric acid, together with other reagents, produces a play of colours with sterol substances. One of these reactions is Schultz test, an application to histology of the Liebermann Burchardt reagent.

Schultz test was applied to all types of placentae examined in this study, but not a single placenta showed the characteristic green colour in the syncytium.

Indophenol oxidase and succinic dehydrogenase reactions were not carried out in this study.

All the above-mentioned reactions were positive in the hands of Wislocki and Dempsey, so they tentatively assumed that syncytial fat represents placental steroid hormones.

As long as there are no specific histochemical methods for the steroid hormones, one has to seek confirmation from chemical procedures.

Tissue culture experiments so far done did not help much in the localisation of placental steroids. Jones and associates in 1943, reported that they were able to identify chorionic gonadotrophins in their tissue cultures, but did not find oestrogen and progesterone in significant amounts. Friedheim (1929) and Sengapta (1935) reported that the syncytium degenerated in their tissue cultures, whereas the cytotrophoblast proliferated abundantly. This is a proof that chorionic gonadotrophins are a product of the cellular trophoblast, but is no confirmation as to what the syncytium relates to the placental steroids and to chorionic gonadotrophins.

However, if tissue cultures are improved to provide ample syncytial growth, one might be able to confirm whether the syncytium is the seat of placental steroids or not.

The other plausible theory about the nature of syncytial fat, is that it represents fat that is being transmitted from mother to foetus for the nutrition and growth of the latter.

This theory was championed by Hofbauer in 1905, and had followers in Bondi (1911) and Froboese (1924), but was not advocated by Wislocki and Bennet (1943). There is no reason why we should discard this theory altogether. That syncytial fat has got something to do with the

metabolism of placental steroids can fit nicely with the assumption that syncytial fat is essentially derived from the maternal blood, and after being transferred to the syncytium, can subserve many functions including nutrition of the foetus as well as elaboration of such substances as placental steroid hormones.

The work of Boyd (1935) and of Boyd and Wesson (1935), indicates that lipoid is transmitted to the foetus in the form of phospholipids (and here phosphatases could be involved), and in lesser amounts as free cholesterol and cholesterol esters. These facts put out the previous theory postulated by Wesson himself in 1926, and supported by Needham in 1931, that the foetus might build its own fat from proteins or carbohydrates instead of obtaining fat directly from the mother.

Decidual Fat:

It is known that there is lipemia in normal pregnancy. Bodansky and Bodansky (1952) have reviewed the literature pertaining to this subject.

According to Boyd (1934), the lipemia is due almost entirely to increases in the plasma lipids. He also reported that neutral fat in the plasma increases in the first trimester, while the phospholipid and cholesterol increase in the second.

These changes in the type of fat in the blood of pregnant women seem to be analogous to changes in the type of decidual fat.

Decidual fat in the first trimester seems to be neutral fat of a nutritional function to the growing embryo.

The positive Schultz test noticed in the decidua of normal pregnancy at term, means that there is some cholesterol or a cholesterol-related fat in the decidua, probably a steroid hormone.

Hormonal Relations:

The amount of fat in the decidua seems to be under hormonal control. All investigators agree that decidual fat in normal pregnancy, like glycogen, is most abundant in the first trimester. Fat and glycogen represent readily available and necessary sources of energy for the developing blastocyst and embryo.

Gillman in 1941, in a study of decidual fat in the first half period of pregnancy, found that maximal fat in the decidua occurs at about the 70th day of pregnancy. This time was found to coincide with the time just past the maximal development of the corpus luteum and the peak of excretion of gonadotrophic hormones.

Fat in the Stroma of Chorionic Villi

Apart from fat in degenerating villi, fat was noticed in the stroma of some healthy chorionic villi in normal placentae starting from 20 weeks pregnancy. This fat was doubly refractile and gave a blue-green colour with Schultz test, and stained with Sudan IV even after the digitonin test. A concurrent presence of non-specific esterase was noticed in the sites of this fat.

This finding raises the possibility that the stroma of chorionic villi might be the seat of production of a steroid hormone. It also modifies the common belief that placental steroid hormones are produced in the syncytium, and only the syncytium.

Obviously, more work will need to be done to solve the problem of placental fat, and confirm the sites of formation of steroid hormones in the placenta.

Non-specific Esterase

The non-specific esterase reaction was seen in the syncytium of the chorionic villi, being strongest in the first trimester, then the intensity of the reaction diminished as pregnancy advanced. Thus its behaviour exactly coincides with that of syncytial fat. It was also seen in the stroma of chorionic villi which showed fat. In the decidua, it was seen in some decidual cells and glands where fat was encountered. These findings

reinforce the observations previously mentioned in the non-pregnant endometrium and strongly suggest that non-specific esterase is concerned with fat metabolism in these tissues.

The reaction was also seen in the lining of blood vessels of the decidua and within their lumena all through pregnancy. To explain this latter finding, one might suppose that such substances are normally present in the maternal blood. It would not be strange then, especially if it is remembered that amongst the non-specific esterase group is choline esterase, which is a normal constituent of blood plasma.

Mucinous Substances

Mucous detectable with the mucicarcime stain was not seen in the normal placenta, apart from the mucous secretion of decidual glands early in pregnancy.

Wislocki and Dempsey in 1948 found metachormatic mucopolysaccharides in the following sites:

A. In the Foetal Placenta:

1. In the ground substance of the umbilical cord,
2. In the coats of umbilical blood vessles,
3. In large placental vessels, and
4. In the stroma of the surface of the placenta.

The stroma of the placental villi was negative except in the fibrous remnants of degenerated villi, and at the

placental labyrinth between the villi and the cytotrophoblastic cell columns.

B. In the Decidua:

They found metachormatic poylsaccharides in the following localities:

1. In the interstitial cell matrix of the decidua.
2. In the secretion of uterine glands.
3. In the ground substance of the walls of the spiral endometrial arteries.

The function of mucopolysaccharides in the decidua is considered nutritive to the growing blastocyst, and also serves as a barrier against the invasiveness of the trophoblast effected by its proteolytic substances.

Calcium

Calcium in the form of phosphate or carbonate, as revealed by Von Kossa method, is seen as very fine black threads beneath the syncytium and in the stroma of chorionic villi all through pregnancy. Coarser calcium deposits are normally found in the placenta at or near term. In such normal cases, the deposits are small and infrequent, and placed essentially at the junctional zone which is the main seat of degeneration in the process of ageing.

The fine stromal deposits represent calcium absorbed from the maternal blood stream, on its way to the foetal blood vessels. This is probably why the reaction is evident in the small tertiary villi, adequately bathed with maternal blood.

Healthy decidual tissues do not show a reaction for calcium.

It is obvious therefore that the foetus gets its calcium directly from the maternal blood, and as the need for calcium in building the foetal skeleton comes pretty late from the onset of gestation, there is no urgent need for calcium before the full establishment of the foetal-maternal circulation in the placenta.

Normal Ageing of the Human Placenta

We have seen that normal ageing of the human placenta starts from about the twenty-eighth week of pregnancy, and as pregnancy advances, the ageing process becomes more and more manifest.

Ageing criteria in the normal placenta could be summarised as follows.

A. Morphological Age Changes:

1. Degenerative changes in the decidua including minor vascular changes and formation of the so-called fibrin layer of Nitabuch.
2. Fibrin Nodes; these are the result of hyalinisation of the stroma of some villi, or of fibrin deposition in the blood spaces between the villi. The neighbouring villi, with the loss of parts of adjacent syncytium and fibrin deposition within and in between them, may be matted together with irregular hyalinisation.

3. Tenney's Nodes: These are formed of aggregations of nuclei in the syncytium. In such nodes, the nuclei are deeply basophilic, with clumping of their chromatin.

4. There is increased deposition of collagen within the stroma of the villi which becomes less cellular. The size and texture of the chorionic villi is more or less uniform.

5. The syncytium is reduced in thickness, and according to Wislocki and Bennet (1943), its brush border and cytoplasmic streamers become simplified.

6. There is swelling and degeneration of mitochondria, and granular appearance of the cytoplasm. This was reported by Dempsey in 1953 as a sign of ageing of the placenta in guineapig using the electron microscope.

B. Histochemical Age Changes:

1. Alkaline and acid phosphatase increase in the syncytium.

2. Ribonucleoprotein, metabolic fat (not degenerative) and non-specific esterase diminish.

3. Fat is increased only in the degenerated areas of the decidua and foetal placenta.

4. Calcium depositions in hyalinised areas is increased while the metabolic calcium is diminished, (calcium transmitted from mother to foetus).

THE PATHOLOGICAL PLACENTA

I. The Placenta in Post-maturity

Note on Histology

The general histology of the post-mature placenta denotes advanced ageing, evident in the increased hyalinisation of chorionic villi and the big collections of calcium deposits. The decidua as well shows more extensive degeneration in the junctional zone than is seen in the normal placenta at term.

Histochemical Changes:

1. Phosphatases:

There is marked increase of alkaline and acid phosphatase reactions in the syncytium of chorionic villi.

2. Ribonucleoprotein:

Ribonucleoprotein in the syncytium is hardly seen in the post-mature placentae.

3. Glycogen:

Glycogen, on the whole, is less than in the normal term placenta.

4. Non-specific Esterase:

There is diminution of non-specific esterase in the syncytium, while the stroma of chorionic villi was completely devoid of that enzymic reaction.

5. Fat:

Degenerative fat is increased, but no fat could be seen in the healthy chorionic villi, neither in the stroma nor in the syncytium.

6. Calcium:

Apart from the calcium deposits in hyalinised areas, the fine stromal reaction indicative of calcium transferred from mother to foetus was hardly seen.

Comment:

Phosphatases:

The increase of alkaline and acid phosphatases in the syncytium of healthy chorionic villi, as in all conditions of ageing of the placenta, might be considered a compensatory phenomenon, as some villi are put out of function. One cannot really tell whether the increase of phosphatases in the syncytium is due to a raised level of these enzymes in the maternal blood, or because these enzymes are formed in excess in the syncytium in such conditions.

According to Bodansky (1939 and 1952), alkaline phosphatase steadily increases in the maternal blood plasma during the progress of normal gestation.

In the literature reviewed, however, no reference was made to changes in plasma phosphatase levels in post maturity.

Ribonucleoprotein:

The depletion of ribonucleoprotein from the syncytium means that protein synthesis is coming to an end.

Glycogen:

The diminution of the glycogen content of the placenta as well as of its oxygen consumption was considered by Claude Villee in 1956 among the criteria of metabolic ageing.

Non-specific Esterase and Fat:

Fat and non-specific esterase could hardly be detected in the stroma and in the syncytium of apparently healthy chorionic villi. Such places were suspected to be the seats of the placental steroid hormones. If this be true, one would expect the placental steroids to diminish in post-maturity. This needs confirmation by chemical assays of placental hormones. I am not aware of any study of the hormonal levels in post-maturity.

The increase in degenerative fat could be taken as a sign of advanced ageing of the placenta.

Calcium:

It is known that calcification of the foetal skeleton goes on beyond term; this means that the foetus is still gaining calcium from the mother. The absence of calcium from the stroma of shorionic villi, therefore, is only indicative of deficient transfer through the syncytium, with consumption of all the available calcium by the foetus.

Conclusion:

All the described histological and histochemical changes in the post-mature placenta seem to be an exaggeration of the normal ageing process due to prolongation of pregnancy.

The cause of normal ageing is thought to be due to relative impairment of maternal circulation through the placenta. This is thought by Reynold (1949) to be the result of increasing intra-uterine pressure and tension within the uterine contents.

As a result of the exaggerated ageing process in the post-mature placenta, its physiological capacities are encroached upon.

It is known that in post-maturity there is impairment of the transfer of substances from mother to foetus; examples are:

1. Oxygen saturation of the cord blood in post-maturity is considerably lower than in normal term pregnancies (Walker and Turnbull, 1953; MacKay, 1957). This, however, was denied by Bancroft-Livingston and Neill in 1957.
2. Sodium clearance rate from the uterus was found to be diminished in post-maturity especially in primigravidae (Moore and Myerscough, 1957).

These examples confirm the explanations suggested for the histological and histochemical pattern of the post-mature placenta.

II. The Placenta in Pre-eclampsia and Eclampsia

The morphological and histochemical change in pre-eclamptic and eclamptic placentae will be discussed under two headings:

A. Changes in the decidua

B. Changes in the foetal placenta.

A. Changes in the Decidua

Histological Changes:

1. Increased hyalinisation at the junctional zone, or in other words, broadening of the so-called fibrin layer of Nitabuch.
2. Increased calcium deposition in the decidua especially in the hyalinised areas.
3. Vascular Pathology:

   In the material of this study, it was not possible to make a survey of the pathological appearance of decidual blood vessels, as the placenta after-birth contains only some remnants of decidua on the maternal surface. Nevertheless, some decidual arteries were met with, showing thickening, hyalinisation, and fatty infiltration of their walls.

Lesions of decidual blood vessels in pre-eclampsia and eclampsia were described by several authors, since they have been noted by Williams in 1915 as acute degenerative arteriolitis. Among the reports on that subject are those by Dieckman in 1936, Hertig in 1945, Zeck and Assali in 1950, Paine, in a combined study with Russell, in 1957, and most recently by Dixon and Robertson in 1958.

The latest description (Dixon and Robertson) could be summarised as follows:

With the onset of hypertension, hyperplastic changes appear first in the media and later in the intima. Hyaline changes may supervene depending on the duration and severity of the hypertensive factor. When the onset of hypertension is sudden and its progression rapid, acute degenerative changes in the form of fibrinoid necrosis, acute arteritis and intravascular thrombosis become evident. The degenerative vessels allow diffusion of plasma through their walls with consequent deposition of fibrin in and around the vessels. This arteriolar damage, causes necrosis and infarction of the decidua which frequently involves the large thin-walled and fragile decidual sinusoids

leading to haemorrhage or further infarction.

It is commonly believed that such pathological changes in the decidual blood vessels need not be initiated by hypertension. The vicious circle can be initiated by the mere effect of uterine distension on the uterine circulation (Reynold (1949) - chapter 36). Browne (1958) found that the influence of uterine distension on the genesis of toxaemia can explain why toxaemia is five or six times commoner in twin than in single pergnancies, and why 75% of toxaemic cases occur in the first pregnancy.

Histochemical Changes:

1. A remarkable increase in the amount of fat in decidual cells. Globules of fat were also noticed in the obviously degenerating areas.
2. Glycogen was also increased in the decidua in comparison with normal placentae of the same age.

Why is decidual fat increased?

Boyd in 1935 and 1936 studied the plasma lipids in eclamptic and pre-eclamptic toxaemia. He found that the increase in plasma lipid in toxaemia was variable, but the ratio of phospholipid/total cholesterol was characteristically higher than in normal pregnancy.

The increase in decidual fat in pre-eclampsia and eclampsia might be attributed to increased phospholipid fractions in the maternal blood.

Why is glycogen increased?

We have mentioned that in pre-eclampsia and eclampsia there is a certain degree of anoxaemia in the decidual tissues especially with gross vascular pathology. It was also mentioned in the first part of this study on the human endometrium, that glycogen is spared in conditions of poor vascularisation when glucose utilisation is more favoured.

Changes in the Foetal Placenta:

General Pathology:

The common occurrence of placental infarctions in cases of pre-eclamptic toxaemia is a fact first discovered by Young in 1914.

More recent descriptions and observations on placental infarctions in toxaemia were presented by Falkiner in 1950, and by Bartholomew in 1957. Lenters in 1958 found a direct relation between the severity of the toxaemia and the number of infarctions in the placenta.

This study, however, is more concerned with the apparently healthy tissues of the placenta, than with the clearly infarcted areas.

Other morphological observations on the placenta in pre-eclampsia and eclampsia have been described by Wislocki

and Dempsey in 1948, and more recently confirmed by Paine (1957). Such data were considered to criteria of ageing only differing in degree from the normal terminal regressive changes. At the end of this chapter, it will be seen that there are some differences between the normal ageing process and that in pre-eclampsia and eclampsia.

Histochemical Changes

It has to be remembered that the blocks chosen for histochemical examination were taken from the apparently healthy placental tissues.

Histochemical changes in the foetal placenta in pre-eclampsia and eclampsia will be discussed under two headings:

A. Changes in the syncytium.

B. Changes in the stroma of chorionic villi.

A. Changes in the Syncytium

Enzymes

There is marked increase in alkaline phosphatase, acid phosphatase, and non-specific esterase, than in a normal placenta of the same age.

Ribonucleoprotein

Ribonucleoprotein of the syncytium is less in amount than in the normal placenta of the same age.

Fat

Metabolic fat in the syncytium is greatly diminished.

Comments

1. The increase of phosphatases and diminution of fat and ribonucleoprotein in the syncytium of pre-eclamptic placentae was previously noticed by Wislocki and Dempsey in 1946. These changes were considered features of premature ageing.
2. It could be noticed that whereas non-specific esterase of the syncytium diminishes to traces in the normally ageing placenta, appreciable amounts are found in pre-eclampsia, while syncytial fat is diminished.

It was assumed in discussing the normal placenta that non-specific esterase, known to hydrolyse esters of fatty acids, is concerned with fat metabolism in the placenta, since it had the same sites as those of placental fat. This assumption can still hold, if we consider the possibility that this enzymic reaction can be produced by several specific enzymes. If the enzyme concerned with the metabolism of syncytial fat is diminished, there is no reason why another specific esterase should not increase.

A non-specific esterase reaction could also be given by choline esterase. Specific substrates for biochemical demonstration of true and pseudo

cholinesterases were used by Thomson (1950), who showed the presence of true cholinesterase in the placenta.

True cholinesterase is an enzyme capable of destroying cholinergic transmitting substance. Its increase might contribute to the rise of blood pressure in toxaemia, in addition to the claimed effect of diminishing placental amine oxidase as a result of low oxygen tension.

Woodbury in 1945 found that placental cholinesterase was increased in pre-eclamptic patients.

It would be very significant, therefore, if the specific substrate for true cholinesterase could be adapted to histology. This, probably, would yield more precise and accurate information than is given by the use of the substrate for non-specific esterase.

3. Syncytial fat, considered by Wislocki and Dempsey to represent placental ketosteroids, was seen to diminish in pre-eclamptic and eclamptic placentae.

It is known that pregnanediol excretion falls in pre-eclampsia (Russell, 1957 and Loraine, 1958).

On the other hand, urinary oestrogen excretion is known to be diminished in the serious forms of pre-eclamptic and eclamptic conditions (Smith and

Kennard, 1937; Walts and Adair, 1943; and Lenters, 1958).

This seems in favour of the theory of Wislocki and Dempsey that syncytial fat has got some relation to placental steroid hormones.

4. It is agreed upon that chorionic gonadotrophins are elaborated in the cellular trophoblast. The possibility of a relation between ribonucleoprotein and placental gonadotrophins was mentioned in discussing the normal placenta.

In severe pre-eclampsia, urinary and serum chorionic gonadotrophins were found to be much more increased than in normal pregnant women (Loraine and Matthew, 1950). The diminution of syncytial ribonucleoprotein in pre-eclampsia does not contradict the fact that chorionic gonadotrophins are elaborated in the cellular trophoblast, although it weakens the possibility that it is also made by the syncytium.

B. Changes in the Stroma of Chorionic Villi

1. Stromal Fat

Fat was seen in the stroma of degenerated villi.

In healthy villi, fat was absent from the secondary (medium sized) villi, but was particularly and constantly found in the stem villi beneath the syncytium, and in the

small villi adjacent to them.

Fat in all sites of the foetal placenta showed no birefringence, and was negative to Schultz test.

The Schultz positive fat which was noticed in the stroma of some chorionic villi in normal pregnancy, was never encountered in placentae of pre-eclamptic or eclamptic patients. In the normal placenta the possibility was mentioned that this fat might represent some steroid hormone that cannot be named for certain. This hormone, therefore, must be absent or diminished in pre-eclamptic and eclamptic placentae.

In this respect, it is felt that an independent detailed study should be carried out for the specification and distribution of steroid hormones in the placenta. This would entail trials for adaptation to histology of the methods now used in biochemical estimation of these hormones, as well as making use of all the properties of these hormones which can show in tissue sections.

The presence of fat in degenerating villi, especially at the periphery of an infarction, is a mere manifestation of fatty degeneration in poorly vascularised areas.

There remains the explanation of the presence of fat in the stem villi beneath the syncytium, and in the small villi adjacent to them. In a study of foetal placental circulation, Crawford (1956) reported that the concentration of capillaries in the cotelydon altered the maternal

blood flow, so that the least resistance to the flow would be near the stem of the cotelydon. The stem villi, therefore, would be more readily and adequately surrounded with maternal blood than the rest of the villi at the periphery of the cotelydon. We can assume, therefore, that the increase of syncytial fat in the stem villi and their adjacent small villi, is due to increased phospholipid fractions in the maternal blood in toxaemias of pregnancy (Boyd).

2. Glycogen

In the foetal placenta of toxaemia glycogen was abundant in the stroma of the stem villi and in the walls of the large blood vessels in these villi. Glycogen was also present, but in small amounts, in the stroma of some of the secondary chorionic villi. It was previously mentioned that glycogen as well as fat was increased in the decidua in pre-eclampsia. But why is there such increase of glycogen in the decidua and in the stem villi in toxaemia? And can this be related to some change in carbohydrate metabolism in that condition?

In normal pregnancy there is increased production of cortical metabolites including glucocorticoids, resulting in a lag curve for sugar tolerance, but no excessive retention of carbohydrate (Hugget, 1950; Browne, 1958).

The action of glucocorticoids (Wright) is in some respects opposite to the action of insulin, thus they raise blood sugar and promote neoglucogenesis but, like insulin, they also increase the deposition of glycogen in the liver. In a similar manner they might influence the deposition of glycogen in the decidua and placenta.

The increased glycogen content of the decidua and placenta in toxaemia might therefore be due to an over-action of glucocorticoids in this condition. This over-action might be due to increased production of placental corticotrophins.

3. Calcium

Deposits of calcium are prematurely laid down in the fibrinous areas of the pre-eclamptic placentae, within the chorionic villi, in between them, and in the margin of the decidua.

Calcium deposition seems to be a sign of sluggish circulation, and it has been previously noticed in fibrin deposits in toxaemic placentae by several authors including Hertig.

Histochemical Age Changes in Pre-eclamptic and Eclamptic Placentae

The histochemical features of normal ageing of the placenta, previously mentioned, were exaggerated in pre-eclamptic and eclamptic placentae.

Such placentae, however, differ by showing the following features:

1. There was increase in the metabolic fat in the decidua and stem villi, as well as increase in the degenerative fat.
2. They showed increase in non-specific esterase activity in the latter period of gestation instead of a decrease in the normal ageing process.
3. Glycogen was increased in the decidua and stem villi.

Conclusion

In the light of the mentioned morphological and histochemical changes in the placenta in pre-eclampsia and eclampsia, it would be easy to understand why the placenta is incapacitated in toxaemia.

Diminished placental function in pre-eclampsia and eclampsia was proved by several procedures; examples are:

1. Diminution of the rate of radio-active sodium transfer from mother to foetus (Browne and Veall, 1953; Moore and Myerscough, 1957).
2. Lowering of the oxygen tension of the venous and arterial blood of the foetus (Walker and Turnbull, 1953; MacKay, 1957).

3. Diminution of oestrogen and pregnanediol excretion in the urine of pregnant women suffering from severe pre-eclamptic toxaemia (known since Smith and Smith in 1934 and verified by several authors mentioned in the previous discussion).

III. The Placenta in Essential Hypertension

Essential hypertension is a generalised vascular disease, so that the spiral arterioles of the decidua are apt to suffer in this disease.

Paine (1957) in his study of the histology of the placenta in normal and abnormal pregnancy described the vascular changes in the decidua and placenta in cases of essential hypertension.

In the decidua, he found thickening of the medial coats of the spiral arterioles, with proliferation of the intima. He sometimes found thrombosis of decidual veins with areas of haemorrhages of variable size and, occasionally, several areas of fibrin deposition in the decidua.

In the foetal placenta, he found the changes compatible with premature ageing, particularly in the vessels of the villous stalks with thickening of the stroma of the chorionic villi due to deposition of coarse adult-type collagen at a premature age of gestation.

The vascular lesion he described as medial thickening and endothelial proliferation, often amounting to oblitera-

tion. He also found no greater degree of degeneration in the syncytium than that of placentae from normal pregnancies of the same age.

Dixon and Robertson in 1958 described similar lesions and reported that the vascular pathology in the placental bed in all hypertensive diseases of pregnancy is essentially the same except in chronic nephritis. A summary of their description was given in the previous chapter on pre-eclamptic and eclamptic placentae.

In this study, which is essentially concerned with histochemical changes, the histochemical pattern of the placenta in cases of essential hypertension showed no deviation from the normally ageing placentae of healthy pregnant women.

Browne and Veall (1953) studying the dispersion of radio-active sodium reported that blood transfer through the placenta was diminished to a greater degree in essential hypertension than in normal pregnancy. The cause of the ischaemia he suggested was primarily extraplacental due to the systemic vascular involvment.

IV. The Placenta in Diabetic Pregnancy

Care was taken to make sure that the pregnant diabetics whose placentae were included in this study had no superimposed toxaemia. From the histological and histo-

chemical findings in the ten placentae examined from diabetic patients, no peculiar pattern can be found for the placenta in Diabetes Mellitus except the increase of decidual fat which can be due to increased blood lipids of the diabetic woman (Bodansky and Bodansky).

Other differences from placentae of normal pregnancy indicate just a process of ageing occurring at an earlier date. In other words, the placenta looks older than its real age so that the term "Diabetic post-maturity" inferred to the foetus of a diabetic mother can also be applied to the placenta in such condition.

The post-maturity of the foetus in diabetic pregnancy is different from the chronological post-maturity. The foetus in diabetic pregnancy becomes excessively large, most probably due to increased production of the growth hormone of the anterior pituitary as Eastman believes (Reis).

The changes in the placenta in Diabetes Mellitus can be due to uterine ischaemia resulting in two ways:-

1. In long standing Diabetes there is usually vascular disease including the decidual blood vessels.
2. Rapid or excessive foetal growth in diabetic pregnancy might lead to ischaemia of the uterine wall, especially in the latter period of gestation.

The interference with the adequacy of the blood supply of the placenta with the resulting age changes in the placental tissues might be a cause of the increased rate of foetal death in diabetic pregnancies.

It has been noted by Russell and co-workers in 1957, that the cause of foetal death in diabetic pregnancy need not be due to placental insufficiency as they sometimes found continued pregnanediol excretion after foetal death in diabetic pregnancy.

V. The Placenta in Chronic Renal Disease

Two placentae were available for histochemical examination from patients with renal failure in whom pregnancy was terminated at 36 and 37 weeks of gestation. One of them was classified as chronic nephritis and the other as type II nephritis.

Comparing with placentae from normal patients at the same period of gestation, the placentae from nephritic patients showed the following differences:-

1. An increase in syncytial alkaline phosphatase.
2. An increase in calcium deposition.
3. A moderate increase in decidual fat.

These histochemical differences from placentae of normal pregnancy might be due to maternal vascular disease or maternal blood changes in these conditions.

The vascular lesion in the placental bed in chronic nephritis is described by Dixon and Robertson (1958) as primarily degenerative with plenty of fibrin leaking from the damaged vessels.

VI. The Hydatidiform Mole

The histochemical observations in the two vesicular moles included in this study resemble to a great extent the results obtained by Wislocki and Dempsey in 1946 in studying the placenta in this pathological condition.

The duration of pregnancy in the two hydatidiform moles studied here did not extend beyond the first trimester, one being of 10 weeks and the other 13 weeks amenorrhoea.

It was noticed that the site of alkaline phosphatase in the trophoblast was not the cytoplasm, as in the normal healthy placentae. It was seen in the cellular membrane, the nuclear membrane and nuclei. This is probably due to a starting process of degeneration.

The amount of alkaline phosphatase and lipid content of the syncytium was not increased more than was seen in the normal placenta of the same age. On the contrary, the amount of ribonucleoprotein in the syncytial and cellular trophoblast, which showed no signs of degeneration, was much increased (compared with a normal placenta in the corresponding period of gestation). This was suspected

by Wislocki and Dempsey to be related to excessive production of chorionic gonadotrophic hormones in vesicular moles.

The increased glycogen in the stroma of some villi which were not yet degenerated and in Langhans' cells, signifies impairment of the circulation and break down in the aerobic mechanisms.

No reaction was seen for calcium with Von Kossa reagent in the chorionic villi where normally it should be seen in the stroma of most of the villi at such an early period of gestation. This was considered by Wislocki and Dempsey to be a sign of impaired transmission by the chorionic villi.

The increase of fat in the decidua can be analogous to its increase in cases of toxaemia.

The presence of needle-shaped, doubly refractile fat crystals which give a positive Schults test in many degenerating villi strongly suggests that this is cholesterol.

SUMMARY

Eight substances were studied by histochemical methods in the human placenta in all stages of normal gestation as well as in certain pathological conditions. The corresponding histochemical patterns were described and discussed.

BIBLIOGRAPHY

Arazac, J.P., and Blanchet, E. (1948): Endocrinol., 8, 315.

Assali, N.S., Hamermesz, J., and Ross, N. (1954): Endocrinol., 55, 561.

Atkinson, W.B. (1950): Menstruation and its Disorders, Engle, P.

Atkinson, W.B., and Elftman, H. (1946): Proc. Soc. Exp. Biol. & Med., 62, 148.

Atkinson, W.B., and Engle, E.T. (1947): Endocrinol., 40, 327.

Atkinson, W.B., and Gusberg, S.B. (1948): Cancer, 1, 248.

Atkinson, W.B., and associates (1949): Cancer 2, 132.

Atkinson, W.B., Gali, E.A., and Gusberg, S.B. (1952): Cancer, 5, 138.

Bancroft-Livingston, G., and Neill, D.W. (1957): J. Obstet. & Gynec. Brit. Emp., 64, 498.

Bartelmez, G.W., and Bensley, C.M. (1932): Special Cytology, 2nd Ed., Cowdry, Paul Hoeber, New York.

Bartholomew, R.A. and associates (1957): Am. J. Obstet. & Gynec., 74, 64.

Bennet, H.S. (1940): Am. J. Anatomy, 67, 151.

Bierry, H. and Gouzon, B. (1936): C.R. Acad. Sci., Paris, 202, 686.

Binder, H., and Neurath, O. (1929): Gebärmutterschleimhaust, Monatschr. F. Geburtsh. U. Gynäk, 81, 170.

Bodansky, M., and Bodansky, O. (1952): Biochemistry of Disease, 2nd Ed., The MacMillan Company, N.Y.

Bodansky, M., Campbell, K., and Ball, E. (1939): Am. J. Clin. Path., 9, 36.

Bondi, J. (1911): Arch. f. Gynack. Berl., XCIII, 189.

Boscott, R.J., and Mandl, A.M. (1949): J. Endocrinol., 6, 132.

Boyd, E.M. (1934): J. Clin. Investigation, 13, 347.

Boyd, E.M. (1935): Bioch. J., 29, 985.

Boyd, E.M., and Wesson, K.M. (1935): J. Clin. Invest., 14, 7.

Boyd, E.M. (1935): Am. J. Obstet. & Gynec., 30, 323.

Boyd, E.M. (1936): Am. J. Obstet. & Gynec., 32, 937.

Brown, J.B., Kellar, R.H., and Matthew, G.D. (1959): J. Obstet. & Gynec. Brit. Emp. April (in press).

Browne, F.J. (1958): The Lancet, Jan. 18, 7012.

Browne, J.C.M., and Veall, N. (1953): J. Obstet. & Gynec. Brit. Emp., 60, 141.

Burton, J.F. (1954): J. Histoch. & Cytochem., 2, 88.

Carleton, H.M. (1938): Histological Technique for Normal Tissues, Morbid Changes and the Identification of Parasites. Oxford Medical Publication.

Casperson, T. (1947): Soc. Exp. Biol., 1, 127.

Cheval, M. (1933-1934): Proc. Roy. Soc. Med., 27, Part II, 1395.

Cohen, R.B., Nachlas, M.M., and Seligman, A.M. (1951): Cancer Res., 11, 709.

Corner, G.W., and Csapo, A. (1953): B.M.J., 1, 687.

Corscaden, J.A., and Gurberg, S.B. (1947): Am. J. Obst. &Gynec., 53, 419.

Crawford, J.M. (1954): J. Obstet. & Gynec. Brit. Emp., 63, pp.87, 542 and 548.

Dempsey, E.W., and Basset, D.L. (1943): Endocrinol., 33, 384.

Dempsey, E.W., and Wislocki, G.B. (1944): Endocrinol., 35, 409.

Dempsey, E.W., and Wislocki, G.B. (1945): Am. J. Anat., 76, 277.

Dempsey, E.W., and Wislocki, G.B. (1946): Physiol. Rev., 26, 1.

Dempsey, E.W., and Wislocki, G.B. (1947): Am. J. Anat., 80, 1.

Dempsey, E.W. (1953): Am. J. Anat., 93, 331.

Dieckmann, W.J. (1936): Am. J. Obstet. Gynec., 31, 734.

Dieckmann, W.J. (1952): Toxaemias of Pregnancy. 2nd. Ed. London, Henry Kimpton.

Dixon, H.G., and Robertson, W.B. (1958): Obstet. & Gynec., 65, 803.

Everett, J.W. (1947): Endocrinol., 41, 366.

Falkiner, N. McI. (1950): The Human Placenta in Toxaemia of Pregnancy. A Ciba Foundation Symposium.

Fujimura, G. (1921): J. Morph., 35, 485.

Friedheim, E.A.H. (1929): Virchows. arch. f. Path. Anat. und. Physiol., 272, 217.

Froboese, C. (1924): Virchow. Arch. Path. Physiol. Klin. Med., 250, 296.

Gey, G.O., and associates (1938): Science, 33, 306.

Gilbert, C. (1942): Endocrinol., 30, 773.

Gillman, J. (1940): Nature, London, 146, 404.

Gillman, J. (1941): South African J. M. Sc., 6. 59.

Gomori, G. (1941a): J. Cell. & Comp. Physiol., 17, 71.

Gomori, G. (1941b): Arch. Path., 32, 189.

Gomori, G. (1946): Am. J. Clin. Path., 16, Tec. Sect. 7, 177.

Greene, H.S.N. and Newton, B.L. (1948): Cancer, 1, 82.

Greene, H.S.N. and Saxton, J.R. Jr. (1938): J. Exper. Med., 67, 691.

Gross, J.S., and Danziger, S. (1957): Am. J. Obstet. & Gynec., 73, 94.

Hagerman, D.D., and Villee, C.A. (1953): Endocrinol., 53, 667.

Hall, J.E., and Brooklyn, N.Y. (1950): Am. J. Obstet. & Gynec., 60, 212.

Hertig, A.T. (1945): Clinics. October, p.602.

Hertig, A.T., and Sommers, S.G. (1949): Cancer, 2, 946.

Hisaw, F.L. (1950): Madison, Wisc., Univ. Wisconsin Press.

Hofofbauer, J. (1905): Anat. Anz. Jena, XXII, 426.

Hugget, A. St.G. (1950): Toxaemia of Pregnancy. A Ciba Foundation Symposium, p.3.

Huggins, C., and Moulton, S.H. (1948): J. Exper. Med., 88, 169.

Hughes, E.C. (1945): Am. J. Obstet. & Gynec., 49, 10.

Jones, G.E. and associates (1943): Bull. J.H. Hosp., 72, 26.

Jones, Howard, W. Jr., and associates (1952): Am. J. Obstet. & Gynec., 64, 1364.

Kabat, E.A., and Furth, J. (1941): Am. J. Path., 17, 303.

Lenters, G.J.W.H. (1958): Oestrioluitscheiding in de Urine en de Anatomischetoe stand van de Placenta Speciaal bij Toxaemie, J.B. Wolters/Groningen.

Lillie, R.D. (1952): Histopathologic Technic. The Blakiston Co., Philadelphia and Toronto, The County Life Press Corp. Garden City, N.Y. pp. 109, 143 and 263.

Lipman, F. (1952): Enzymologia, 15, 251.

Loraine, J.A. (1958): Clinical Application of Hormone Assay. Livingstone (Ed.)

Loraine, J.A., and Matthew, G.D. (1950): J. Obstet. & Gynec. Brit. Emp., 57, 542.

Lorincz, A. and associates (1957): J. Obstet. & Gynec. Brit. Emp., 64, 185.

McKay, D.G. (1957): Progress in Gynecology, Vol. III, p. 3. Meigs and Sturgis, Editors. Grune and Stratton.

McKay, D.G., and Robinson, D. (1947): Endocrinol., 41, 378.

McKay, D.G. and associates (1949): Am. J. Obstet. & Gynec., 58, 625.

McKay, D.G. and associates (1955): Anat. Record., 122, 125.

McKay, D.G. and associates (1956): Obstet. & Gynec., 8, pp. 22 and 140.

Meyer, R. (1922): Arch. f. Gynäk., 115, 394.

Meyer, R.K. and McShan, W.H. (1950): Menstruation and its Disorders. Engle, p.62.

Mishell, D.R., and Motyloff, L. (1941): Endocrinol., 28, 436.

Moog, F. (1946): Biol. Rev., 21, 41.

Moore, P.T., and Myerscough, P.R. (1957): J. Obstet. & Gynec. Brit. Emp., 64, 207.

Nachlas, M.M., and Seligman, A.M. (1949b): J. Nat. Cancer Inst., 9, 415.

Needham, J. (1931): Chemical Embryology. New York, Cambridge, England, The Macmillan Company and the University Press.

Novak, E.R. (1956): Am. J. Obstet. & Gynec., 71, 1312.

Novak, E., and Martzloff, K.H. (1924): Am. J. Obstet. & Gynec., 8, 385.

Novak, E.R., and Mohler, D.I. (1953): Am. J. Obstet. & Gynec., 65, 1099.

Novak, E., and Novak, E.R. (1958): Gynecologic and Obstetric Pathology, 4th ed. W.B. Saunders, Co., Philadelphia and London.

Overholser, M.D., and Nelson, W.O. (1936): Proc. Soc. Exper. Biol. & Med., 34, 839.

Page, E.W. and associates (1951): Am. J. Obstet. & Gynec., 62, 1100.

Paine, C.G. (1957): J. Obstet. & Gynec. Brit. Emp., 64, 668.

Pearse, G.E. (1953): Histochemistry Theoretical and Applied, pp.408 and 437.

Pollock, W.F. (1942): Anat. Record, 84, 23.

Potter, E.L. (1953): Pathology of the Foetus and the Newborn. Year Book Publishers Inc., Chicago.

Reis, R.A. and associates (1952): Diabetes and Pregnancy. Charles C. Thomas Publisher, U.S.A.

Reynold, S.R.M. (1949): Physiology of the Uterus. 2nd. ed. Hoeber.

Rossman, I. (1941): Am. J. Anat., 69, 187.

Russell, C.S. and associates (1957): J. Obstet. & Gynec. Brit. Emp., 64, 649.

Sessums, J.U. and Murphy, D.R. (1933): Surg. Gynec. & Obstet., 56, 500.

Shroeder, R. (1954): Am. J. Obstet. & Gynec., 68, 294.

Simkin, J.L., and Work, T.S. (1957): Nature, 179, 1214.

Skjoldberg, H. (1954): Congres International de Gyn. et d'Obstetrique. Geneve, p.358.

Smith, G.V., and Smith, O.W. (1934): Am. J. Physiol., 107, 128.

Smith, G.V., and Kennard, J.H. (1937): Proc. Soc. Exper. Biol. & Med., 36, 508.

Sommerville, Ian F. (1950): Toxaemia of Pregnancy. A Ciba Foundation Symposium. p.216.

Speert, H. (1952): Cancer, 5, 927.

Stowell, R.E. (1947): Symp. Soc. Exp. Biol., 1, 190.

Stuermer, V.M., and Stein, R.J. (1950): Am. J. Obstet. & Gynec., 6, 711.

Stuermer, V.M., and Stein, R.J. (1951): Am. J. Obstet. & Gynec., 61, 414.

Stuermer, V.M., and Stein, R.J. (1952): Am. J. Obstet. & Gynec., 63, 359.

Sumner, J.B., and Somers, F. (1953): Chemistry and Methods of Enzymes. 3rd. ed. Academic Press Inc., N.Y.

Telfer, M.A. (1953): Arch. Biol., 44, 111.

Te Linde, R.W. (1950): Menstruation and its Disorders. Engle. p.250.

Te Linde, R.W. and associates (1953): Am. J. Obstet. & Gynec., 66, 952.

Tenney, B. and associates (1955): Am. J. Obstet. & Gynec., 70, 889.

Tenney, B. and associates (1958): Am. J. Obstet. & Gynec., 75, 656.

Thompson, R.H.S. (1950): Toxaemia of Pregnancy. A Ciba Foundation Symposium. p.236.

Veraldo, J.T. (1954): Anat. Record., Suppl. 1, 20, 770.

Villee, C.A. (1956): Colloquia on Ageing, 2, 129.

Walker, J., and Turnbull, E.P.N. (1958): Lancet 2, 312.

Walker, J. (1954a): J. Obstet. & Gynec. Brit. Emp., 61, 162.

Watts, R.M., and Adair, F.L. (1943): Am. J. Obstet. & Gynec., 46, 183.

Wesson, L.G. (1926): Johns Hopkins Hosp. Bull. Balt., XXXVIII, 237.

Wheeler, J.D., and Danziger, S. (1955): Obstet. & Gynec., 5, 739.

Wied, G.L., and Sechelmann, F.J.K. (1952): Zentralbl. Gynäk., 74, 2033.

Wislocki, G.B., and Bennet, H.S. (1943): Am. J. Anat., 73, 325.

Wislocki, G.B., and Dempsey, E.W. (1945): Am. J. Anat., 77, 365.

Wislocki, G.B. and associates (1950): Menstruation and its Disorders. Engle. p.23.

Wislocki, G.B., and Dempsey, E.W. (1946): Endocrinol., 38, 90.

Wislocki, G.B., and Dempsey, E.W. (1948): Am. J. Anat., 83, 1.

Wislocki, G.B. (1956): Colloquia on Ageing, Ciba Foundation, 2, 105.

Woll, E. and associates (1948): Am. J. Obstet. & Gynec., 56, 617.

Woodbury, R.A. and associates (1945): J.A.M.A., 128, 585.

Young, J. (1914): Proc. R. Soc. Med., 7, Pt. 2, 307.

Zeek, P.M., and Assali, N.S. (1950): Am. J. Clin. Path., 20, 1099.

THE NORMAL ENDOMETRIUM

Photo 1. 1 (1) x 110.

Alkaline Phosphatase. Earlyproliferative endometrium. Paraffin section - Calcium-Cobalt Method. The reaction is represented by the black colour in glandular and surface epithelium. It is also seen in the endothelium of blood vessels.

Case No.4998/57.

Photo 2. 1 (2) x 110.

Alkaline Phosphatase in late-proliferative endometrium. Paraffin section - Calcium-Cobalt Method. The reaction is more intense than in the mid-proliferative endometrium. In the glands, it can be seen in the lumens.

Case No. 2584/57.

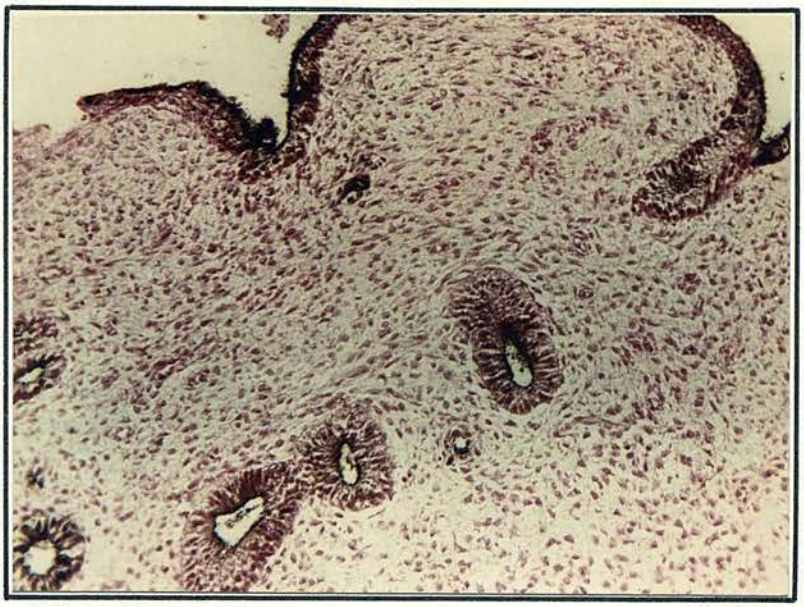


Fig. 1 X 110

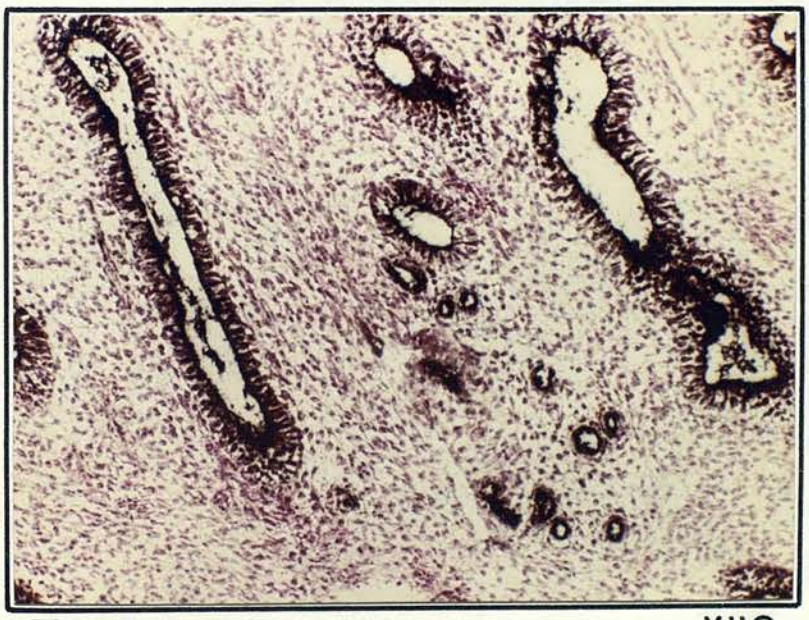


Fig. 2 X110

Photo 3. 1 (3) x 110.

Acid Phosphatase in late proliferative endometrium. Frozen section. Azo-Coupling Method. The black colour which represents the enzymatic activity is present in few glands in the cytoplasm of their epithelium especially at the luminal tips.

Case No. 4136/57.

Fig. 3 X110

Photo 4. 1 (4) x 110.

Non-specific Esterase in mid-proliferative endometrium. Frozen section. Azo-Coupling Method. The activity of the enzyme is represented by a black deposit in 3 sites:

1. The glandular epithelium at the luminal tip in the cytoplasm.
2. The outer border of the surface epithelium also cytoplasmic.
3. A few stromal cells.

Case No. 4998/57.

Photo 5. 1 (5) x 110.

Non-specific Esterase in late-proliferative endometrium. Frozen section. Azo-Coupling Method. The reaction is stronger than in the mid-proliferative phase.

Case No. 4136/57.

Fig. 4 X 110

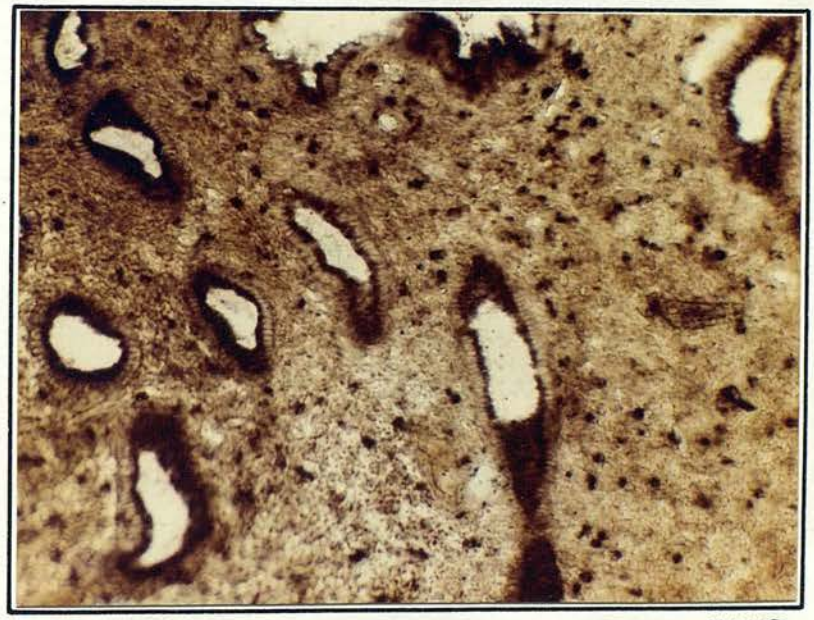


Fig. 5 X110

Photo 6. 1 (6) x 110.

Glycogen - Chromic Acid Schiff - diluted light green counterstain. Mid-proliferative endometrium showing scanty glycogen in glandular and surface epithelium.

Case No. 4998/57.

Photo 7. 1 (7) x 110.

Mucin in mid-proliferative endometrium. Mucicarmine stain - Mucin is scanty in this phase of the menstrual cycle. In the figure it is only seen in one gland at the luminal tip of the epithelium (red colour).

Case No. 4998/57.

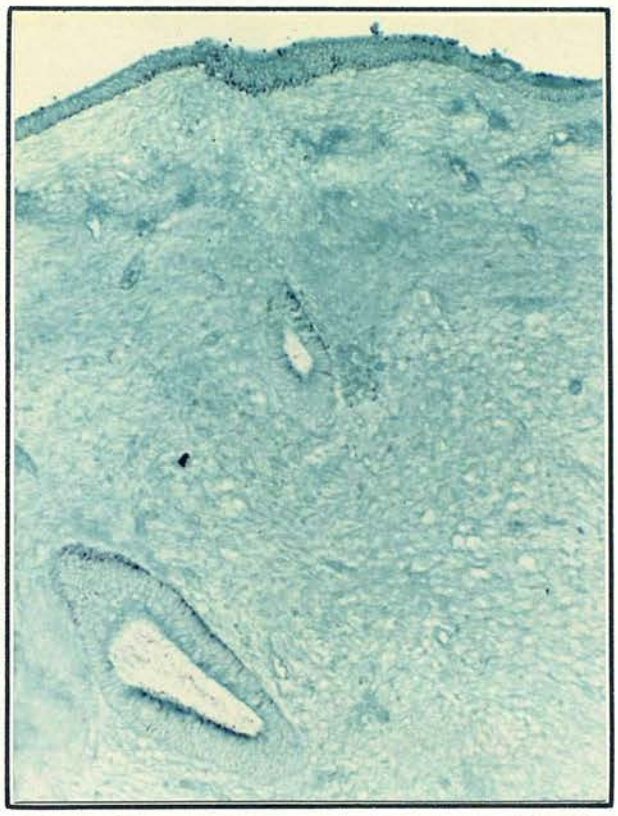


Fig. 6 XIIO

Fig. 7 XIIO

Photo 8. 1 (8) x 110.

Cytoplasmic basophilia in glandular and surface epithelium of endometrium in the early proliferative phase. Eosin Methylene Blue stain. (The red colour of the R.B.C.'s is our guide to the proper point of differentiation.)

Slide No. D. 4303.

Photo 9. 2 (1) x 185.

Marked cytoplasmic basophilia in late proliferative endometrium. Eosin Methylene Blue stain.

Slide No. D.4308.

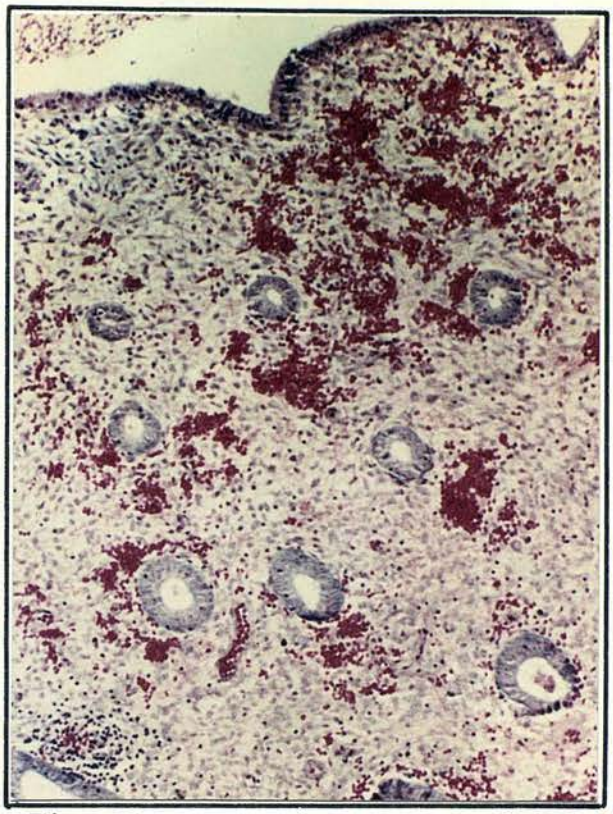


Fig. 8 X110

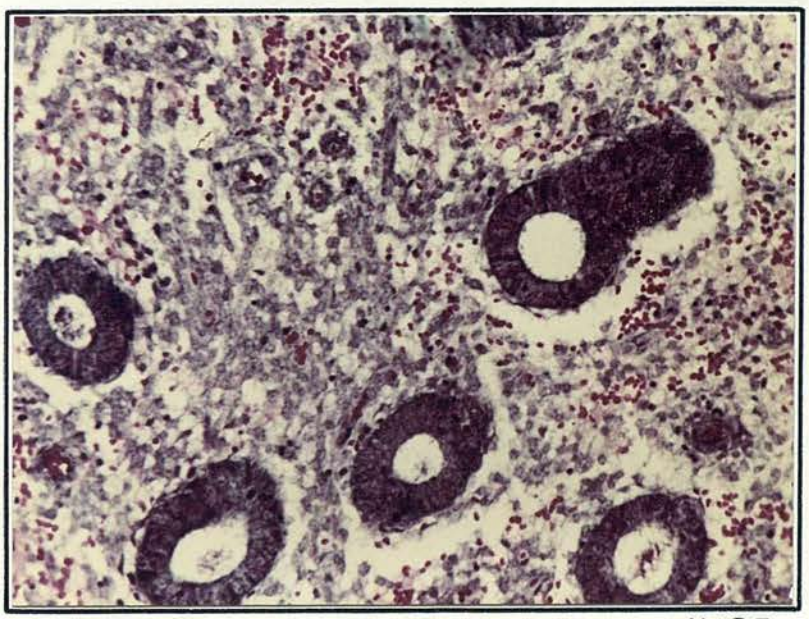


Fig. 9 X185

Photo 10. 3 (3) x 400.

Late secretory endometrium with very slight cytoplasmic basophilia in the glandular epithelium overshadowed by the eosinophilic constituents but evident in part of the glandular secretion within the lumens.

Slide No. 502/57.

Photo 11. 3(4) x 400.

The control section of the above case after treatment with Ribonuclease. Notice that the basophilia inside the lumen has also disappeared.

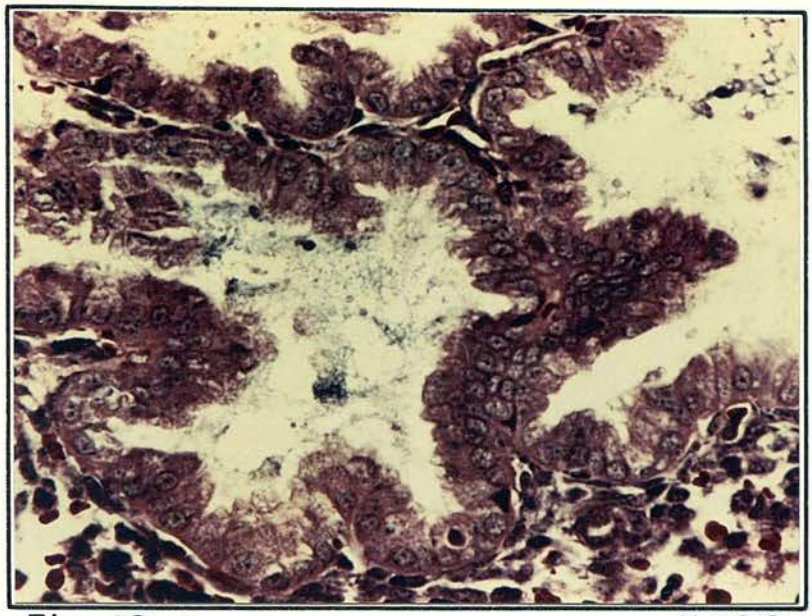


Fig. 10 X400

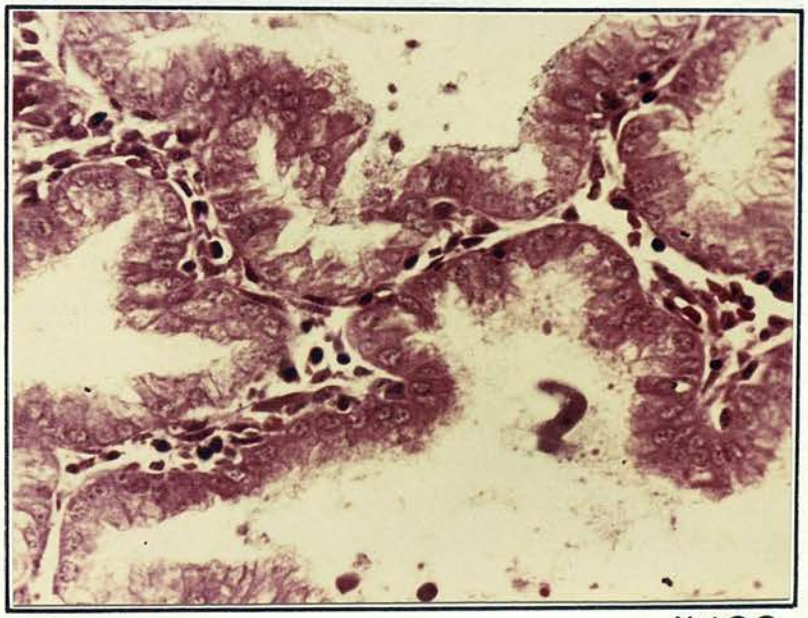


Fig. 11 X400

Photo 12. 3 (2) x 400.

High power magnification of a gland showing cytoplasmic basophilia in early secretory endometrium. Intensity less than that of late proliferative and more than that of late secretory endometrium.

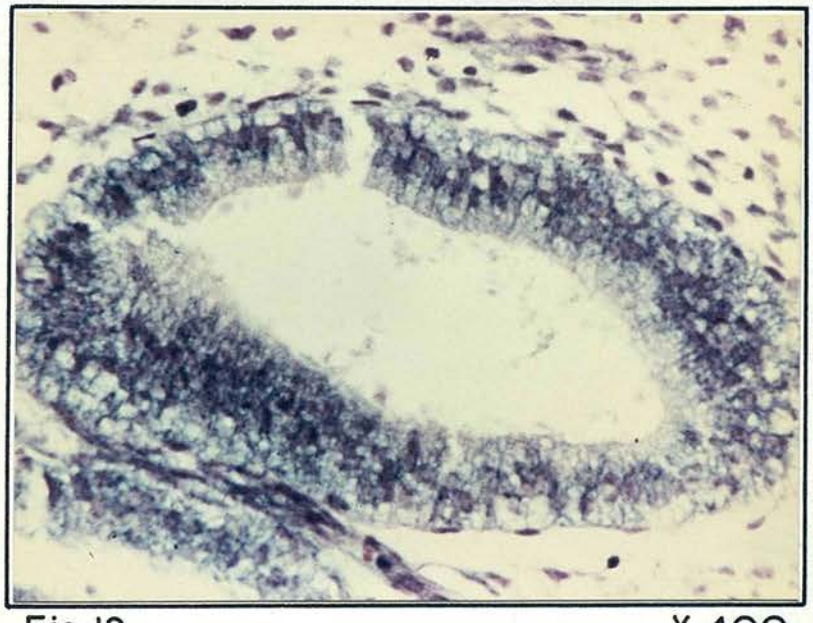


Fig. 12 X 400

Photo 13.

Mid-proliferative endometrium showing neutral fat in the stroma. Frozen section - Sudan IV with dilute haematoxylin counterstain.

Case No. 4998/57.

Photo 14.

Mid-secretory endometrium showing greater increase in the amount of stromal fat than the previous case. Frozen section - Sudan IV with dilute haematoxylin counterstain.

Case No. 5099/57.

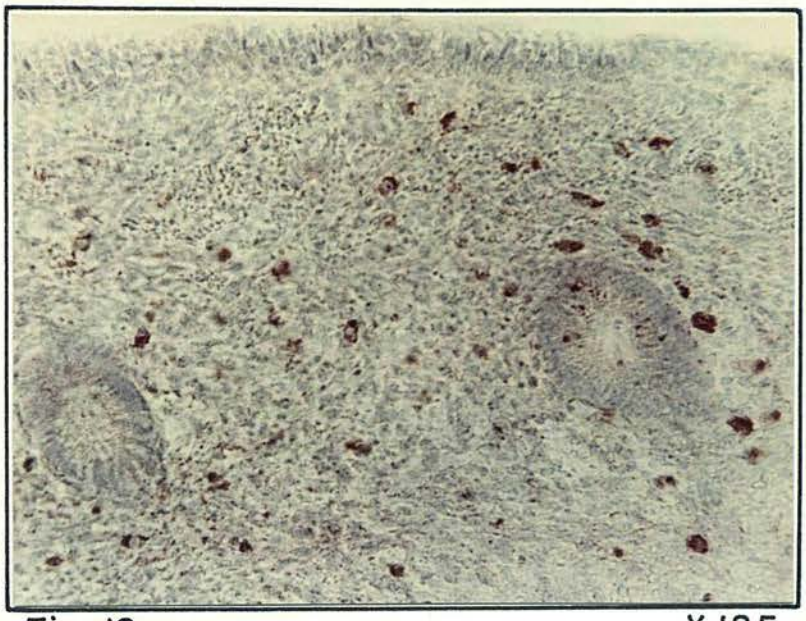


Fig 13 X 185

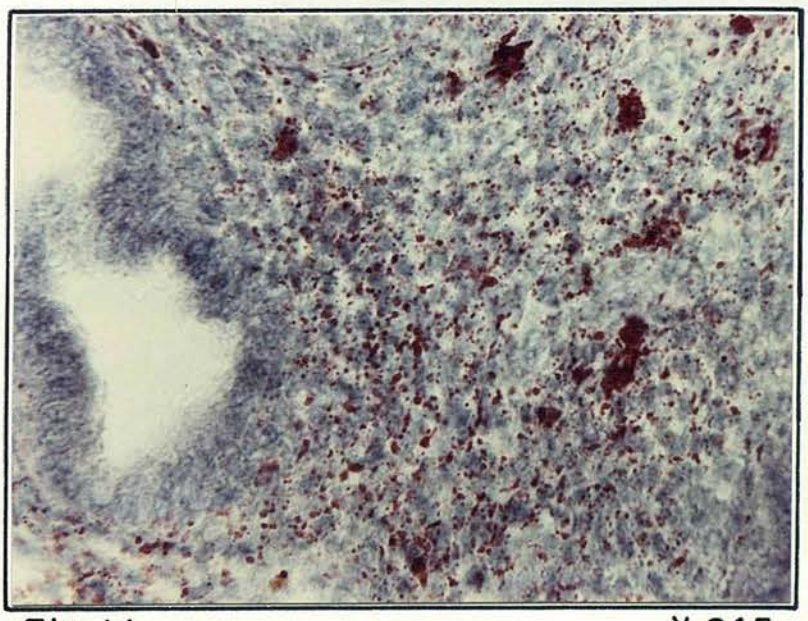


Fig. 14 X 365

Photo 17.

Alkaline phosphatase in mid-secretory endometrium. A slight reaction is present in the glandular epithelium. The blood vessels show a positive reaction in their lining. Frozen section - Azo Coupling Method - haematoxylin counterstain.

Case No. 4255/57.

Photo 18.

Alkaline phosphatase in late secretory endometrium. Besides the blood vessel endothelium, the reaction is present in the lumen of few glands. The figure shows a gland under high power with some blood vessels around. Paraffin section - Calcium Cobalt Method.

Case No. 5695/56.

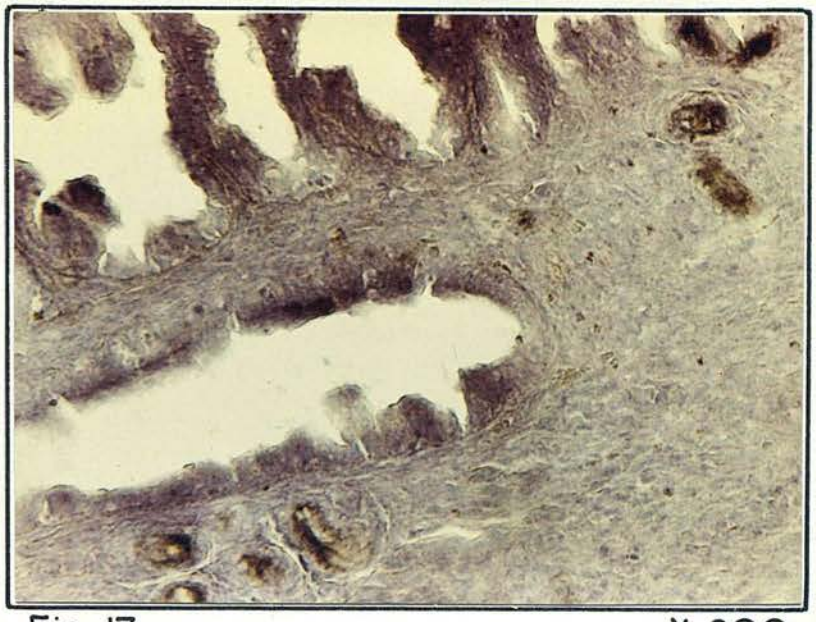


Fig. 17 X 200

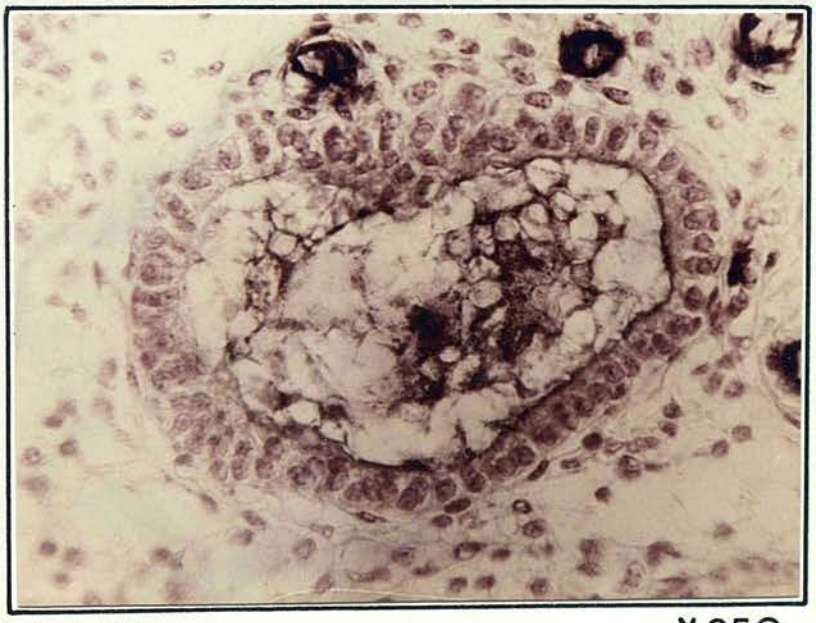


Fig. 18 X 350

Photo 19.

Acid phosphatase in glands of mid-secretory endometrium. A heavy black colour is seen in the glandular epithelium in the cytoplasm particularly towards the lumen (nuclei are basal in this phase of the cycle and could be seen in some parts free from the colour reaction). Some part of the secretion within the lumens shows the black coloured deposits. Frozen section - Azo Coupling Method - No counterstain.

Case No. 5099/57.

Photo 20.

Acid phosphatase paraffin method of Gomori - mid-secretory endometrium. By this method the reacton is only seen in the nuclei of stroma cells. (Reaction black - eosin counterstain.)

Case No.2804/57.

Fig. 19 X120

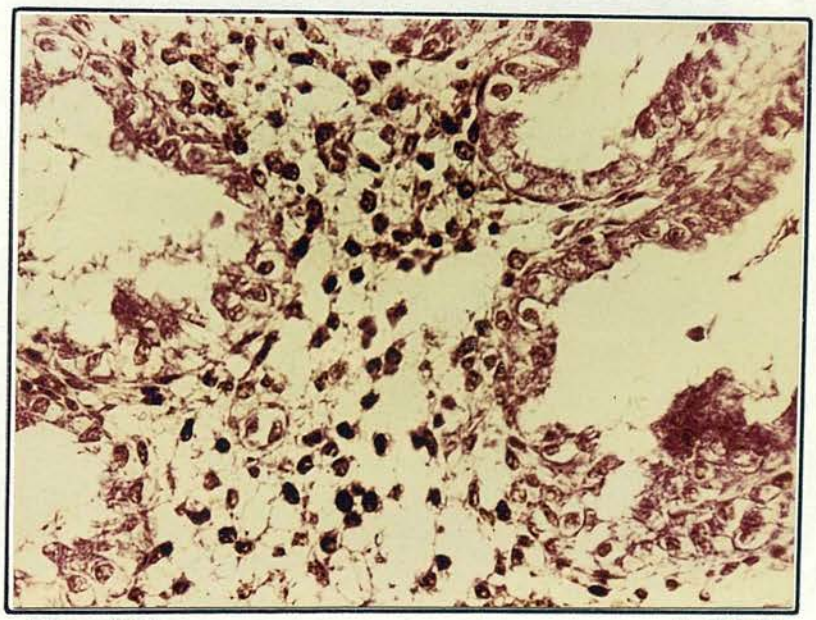


Fig. 20 X 350

Photo 21.

Non-specific Esterase in mid-secretory endometrium. The black colour represents the reaction which is present in three sites:

1. The luminal tip of the cytoplasm of the glandular epithelium.

2. The cytoplasm of the surface epithelium to a lesser degree.

3. In some stroma cells.

Frozen section - Azo Coupling Method. No counterstain.

Case No. 4361/57.

Photo 22.

Mucin in early mid-secretory endometrium. Large quantities of mucin are seen in the lumens of glands, whereas the cytoplasm of the glandular epithelium (which by now is rich in glycogen especially at the base) shows the crimson red colour only at the luminal tips. The surface epithelium also shows a slight colour at the free border - Mucicarmine Stain.

Case No. 3315/57.

Fig. 21 X110

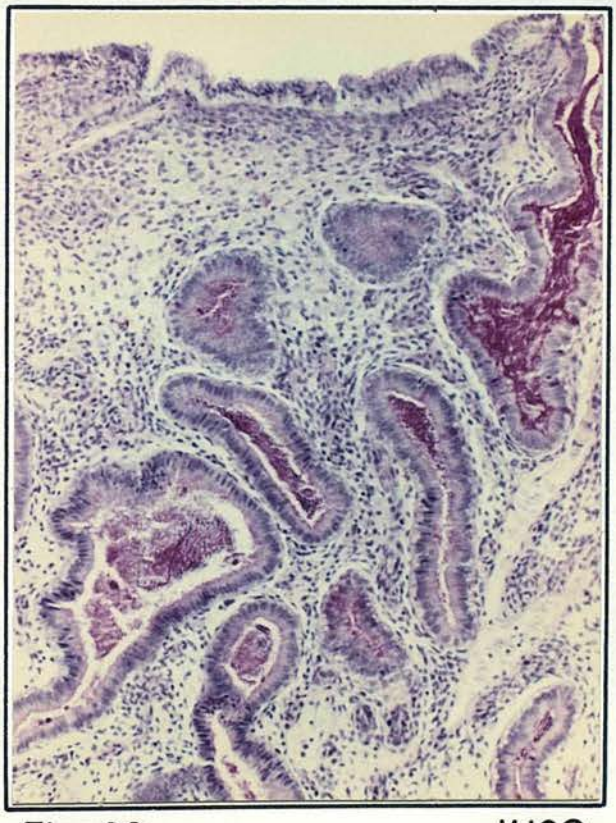


Fig. 22 X120

Photo 23.

Glycogen in mid-secretory endometrium by the chromic acid Schiff Method - dil. light green counterstain - Normally glycogen in this phase of the cycle is to be found in three sites:

1. Glands.
2. Surface epithelium.
3. Stroma cells.

Case No. 479/57.

Photo 24.

Glycogen in the glands of mid-secretory endometrium - Best's Carmine Stain - Ehrlich's haematoxylin counter-stain.

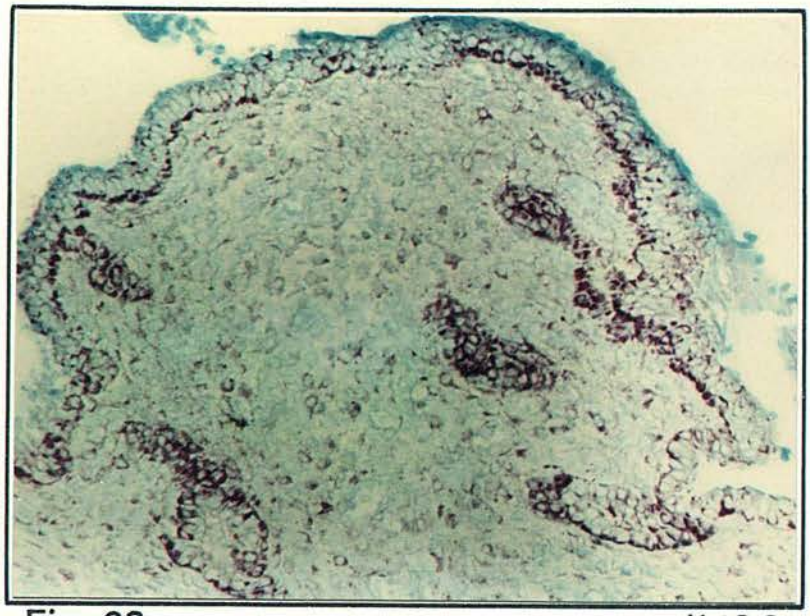


Fig. 23 X 180

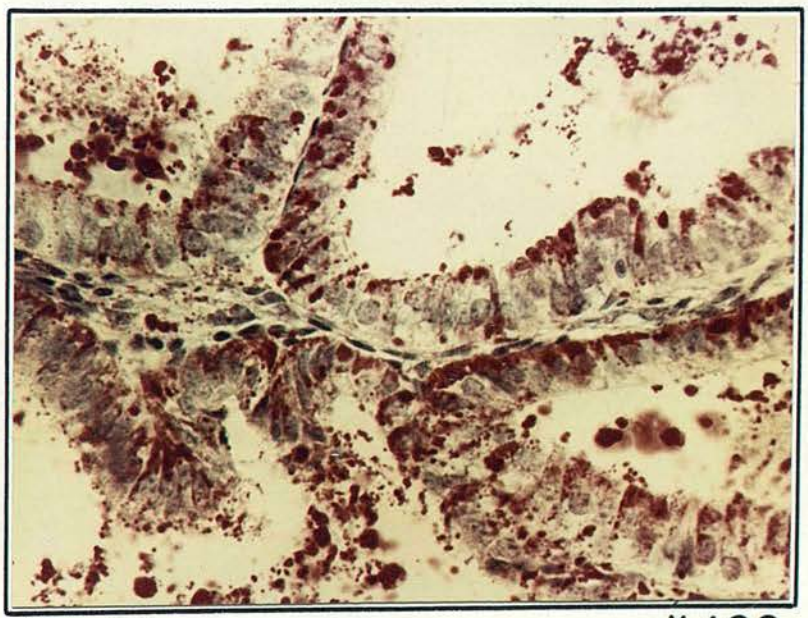


Fig. 24 X 400

METROPATHIA HAEMORRHAGICA

Photo 25.

Alkaline phosphatase in cystic hyperplasia. Enormous amounts in the glandular epithelium at the luminal tips of the cytoplasm and in the lumens.

Frozen section - Azo-Coupling Method. No counterstain.

Case No.2786/57.

Photo 26.

Acid phosphatase absent in this case of metropathia haemorrhagica. There is no black colour deposition in the brown background.

Frozen section - Azo-Coupling Method.

Case No.4045/57.

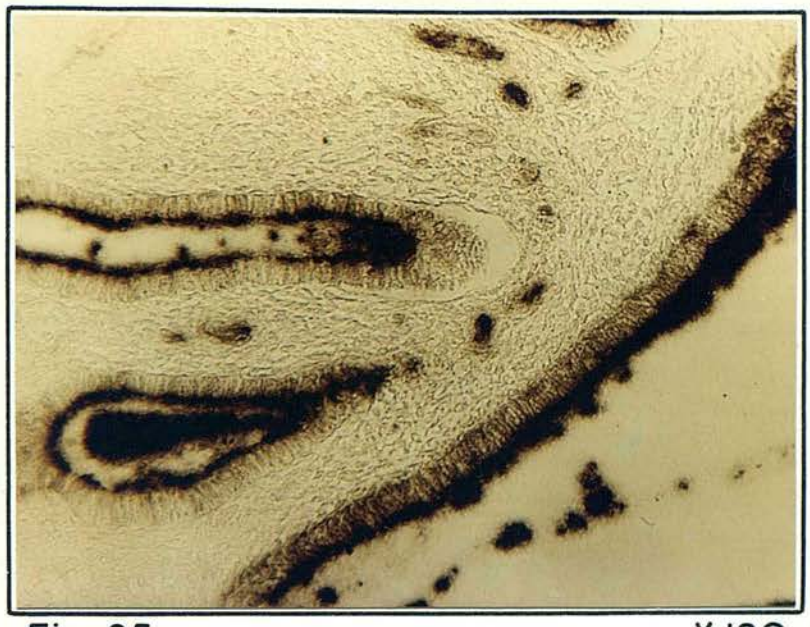


Fig. 25 X 130

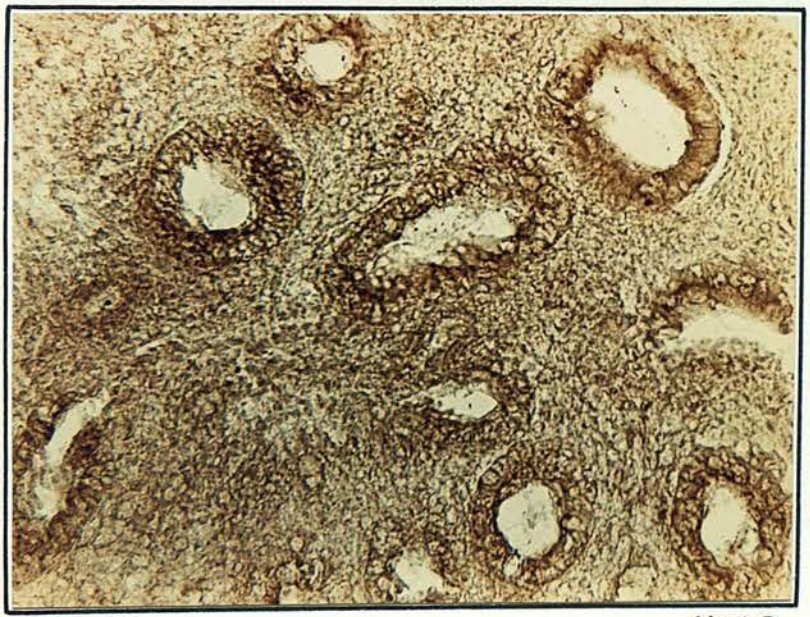


Fig. 26 X 160

Photo 27.

Non-specific Esterase in cystic hyperplasia. The increase in the amount of this enzyme is proportional to the degree of hyperplasia.

Case No. 5860/57.

Frozen section - Azo-Coupling Method. No counterstain.

Photo 28.

Cytoplasmic basophilia in cystic hyperplasia is more than seen in normal endometrium. Eosin Methylene Blue stain.

Slide No. D.4145.

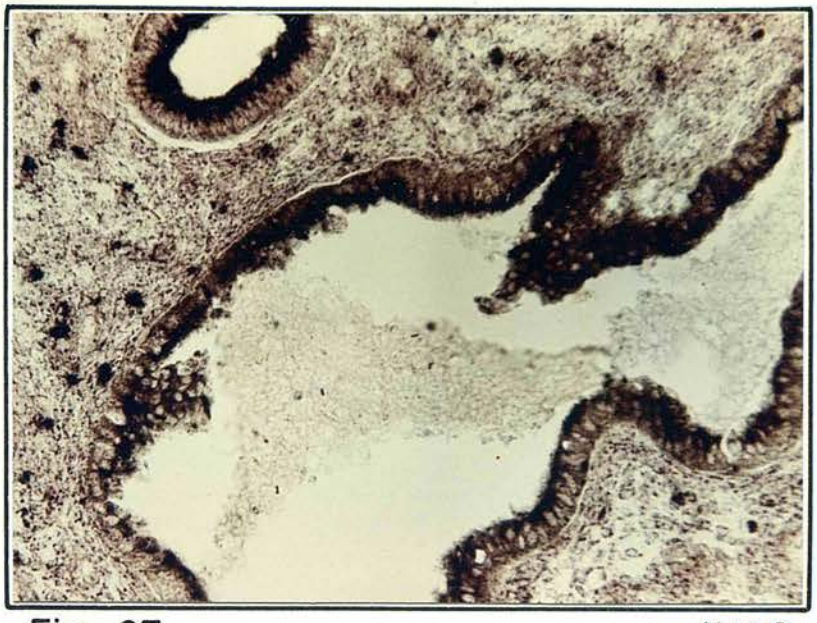


Fig. 27 X160

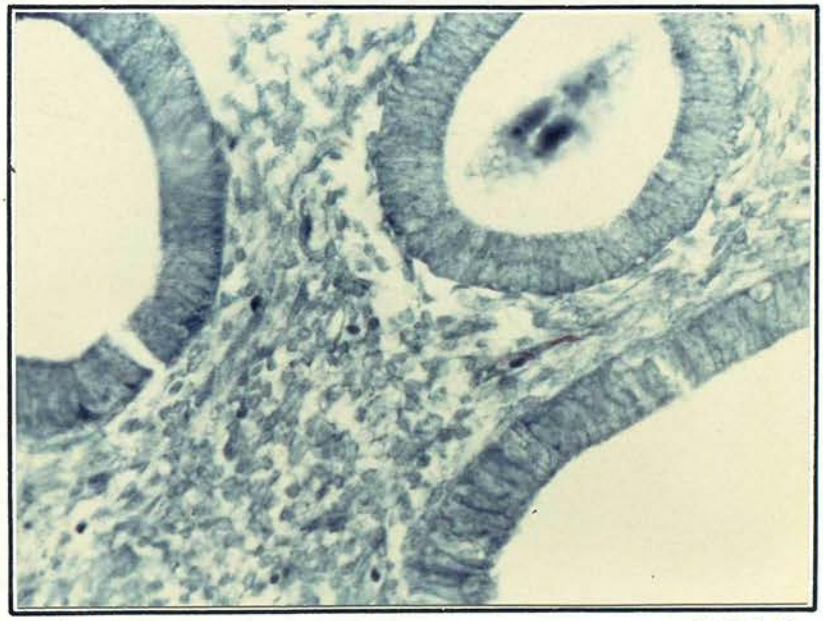


Fig. 28 X350

Photo 29.

Best's Carmine stain shows no glycogen in this figure which represents a genuine case of metropathia haemorrhagica.

Case No. 5860/57.

Photo 30.

Mucicarmine stain showing cystic glands full of mucinous secretion. Metropathia haemorrhagica.

Case No.2217/57.

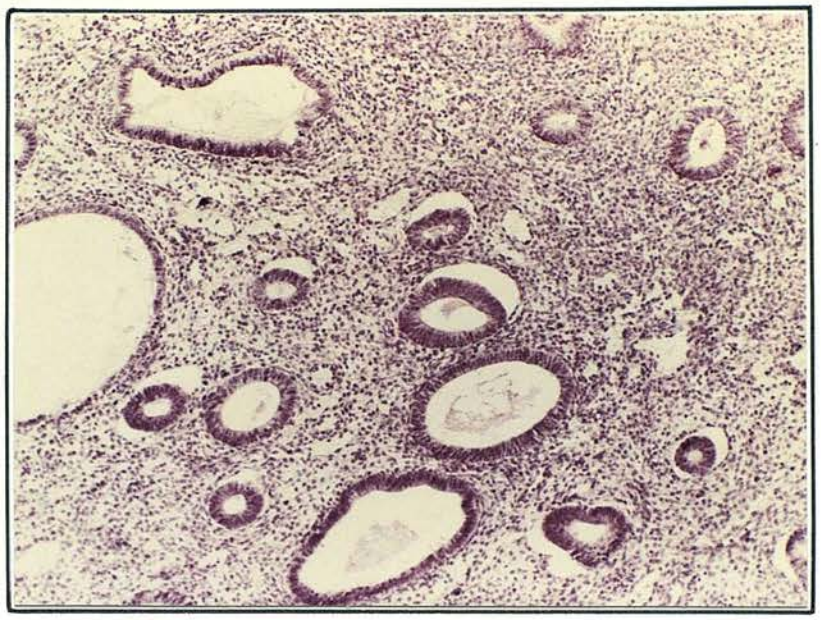


Fig. 29 X 80

Fig. 30 X 90

Photo 31.

Sudan IV stain showing increased stromal fat in cystic hyperplasia.

Case No. 1168/58.

Photo 32.

Stromal fat in cystic hyperplasia of the endometrium, showing double refraction with polarised light.

Case No. 2107/57.

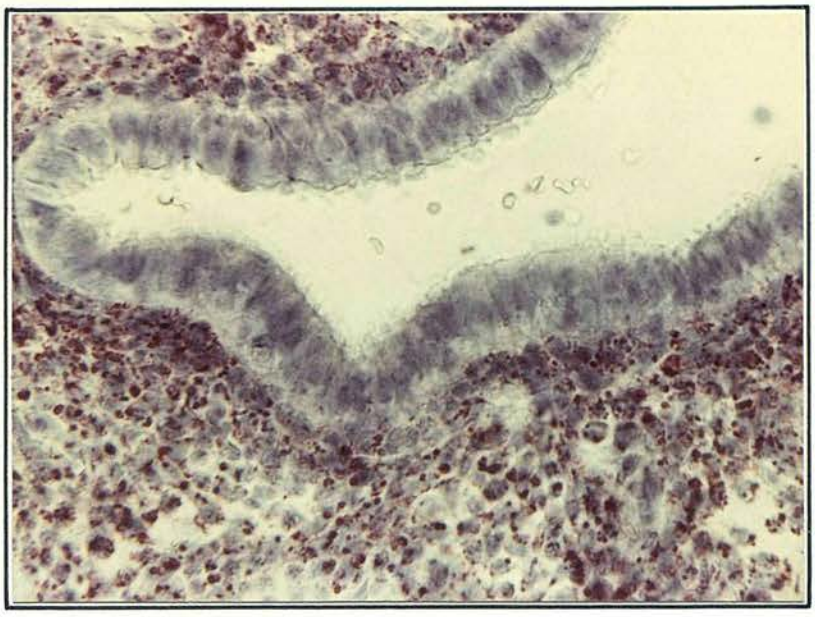


Fig. 31 X350

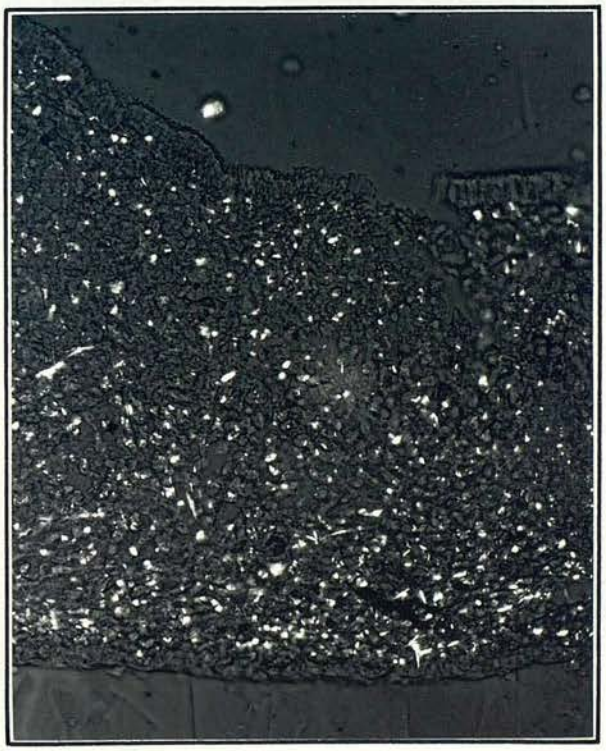


Fig. 32 X 100

Photo 33.

Schultz Test in cystic hyperplasia. The green colour is faint all over the stroma with heavy collections of green coloured material in some areas.

Case No. 1168/58.

Fig. 33 X 175

ENDOMETRIAL CANCER

Photo 34.

Alkaline phosphatase in endometrial adenocarcinoma is only present in blood vessels. Frozen section - Azo Coupling Method - No counterstain.

Case No. 3648/57.

Photo 35.

Alkaline phosphatase in papillary adenocarcinoma of the endometrium, almost only seen in blood vessels. Paraffin section, Calcium-Cobalt Method.

Case No. 356/58.

Fig. 34 X 90

Fig. 35 X90

Photo 36.

Acid phosphatase markedly increased in carcinomatous growth of the endometrium in the epithelial elements. Frozen section. Azo-Coupling Method. No counterstain.

Case No. 2946/57.

Photo 37.

Acid phosphatase in endometrial carcinoma by the paraffin method of Gomori showing a universal nuclear reaction in the glandular and stromal elements.

Case No. 3648/57.

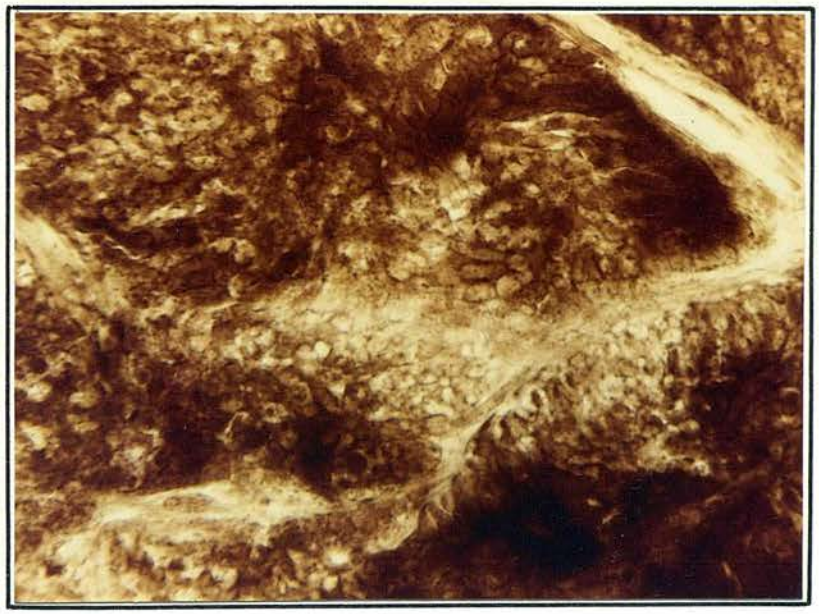


Fig. 36 X 35

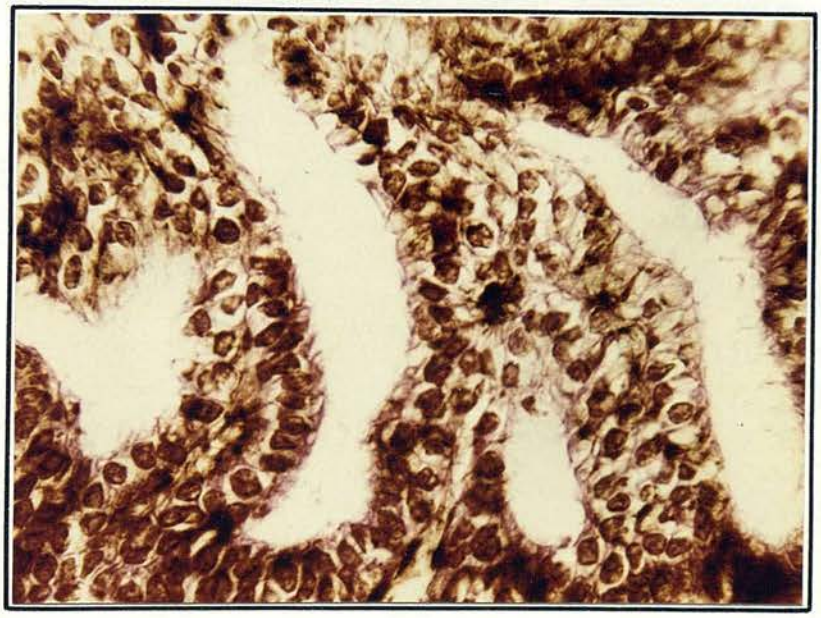


Fig. 37 X 400

Photo 38.

Non-specific esterase in endometrial carcinoma. Marked increase in the intensity of the reaction. Frozen section. Azo-Coupling Method.

Case No. 356/58.

Photo 39.

The same case (356/58) showing increased activity of non-specific esterase even in paraffin sections. (Azo-Coupling.)

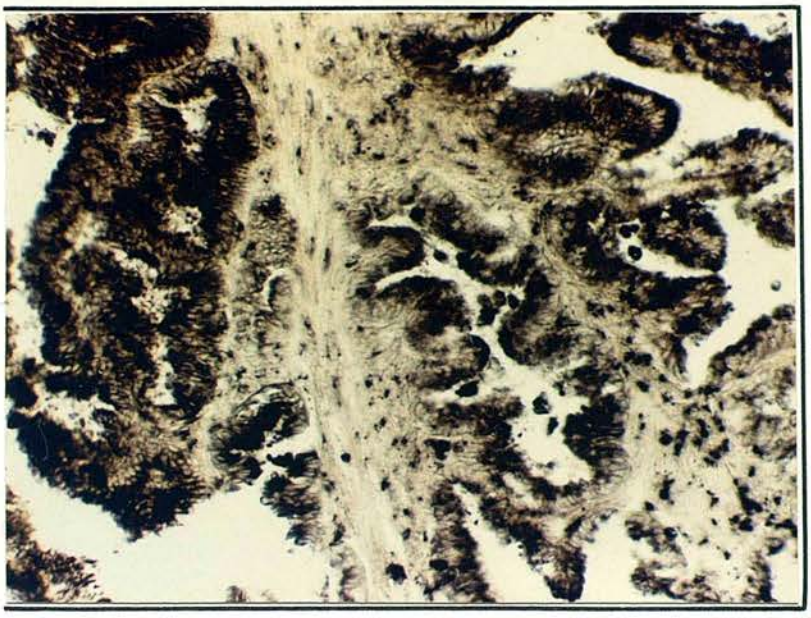


Fig. 38 X 90

Fig. 39 X 165

Photo 40.

Best's Carmine stain showing no glycogen in a case of well differentiated adenocarcinoma of the endometrium.

Case No. 806/58.

Photo 41.

Best's Carmine stain showing heaps of glycogen in a rapidly growing cancer of the endometrium.

Case No. 4887/57.

Fig. 40 X 165

Fig. 41 X 165

Photo 42.

Heavy cytoplasmic basophilia in epithelial elements of papillary adenocarcinoma (this basophilia is abolished after incubation of a control slide with Ribonuclease). Eosin-Methylene Blue Stain.

Case No. 532/57.

Photo 43.

A well differentiated mucous secreting type of cancer of endometrium. Southgate's Mucicarmine Stain.

Case No. 3648/57.

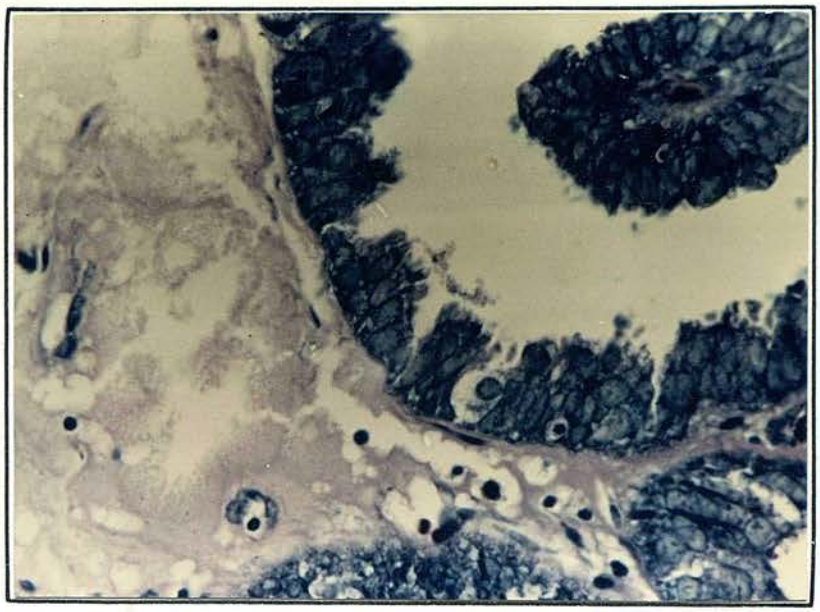


Fig. 42 X 365

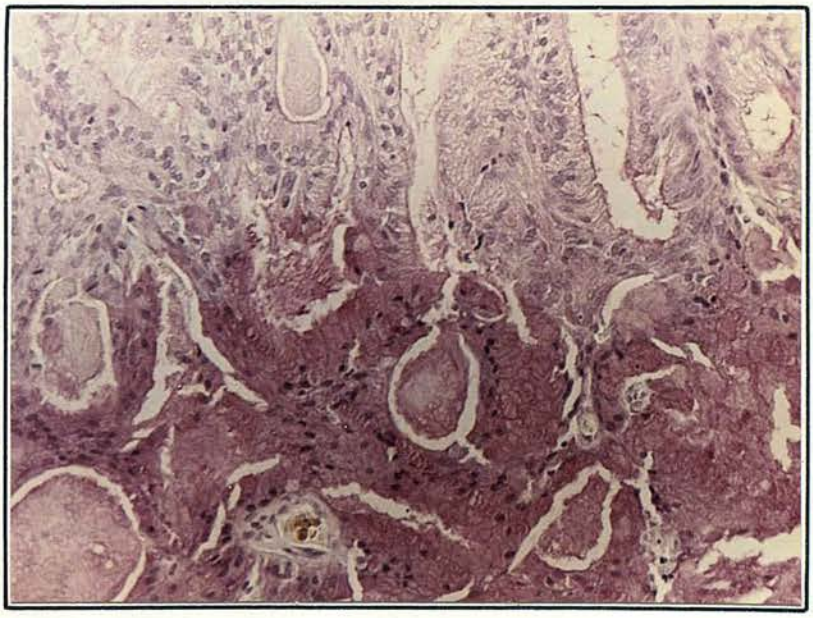


Fig. 43 X 165

Photo 44.

Sudan IV stain showing enormous amounts of fat in the stroma between cancerous glands in the endometrium. The glandular epithelium in some glands shows small droplets of fat.

Case No. 806/58.

Photo 45.

Schultz test is strongly positive in endometrial carcinoma. The intense green colour is seen in sites of maximal accumulation of fat.

Case No. 356/58.

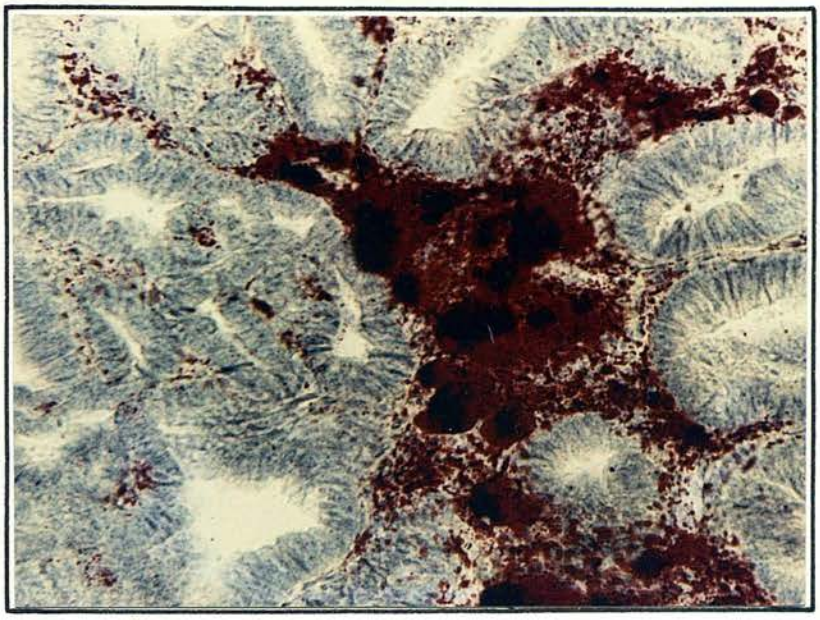


Fig. 44 X 165

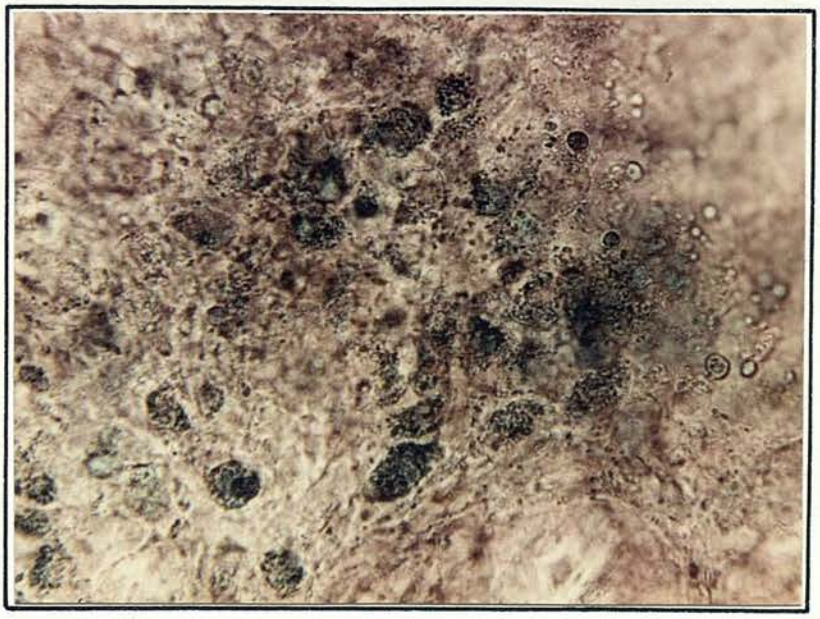


Fig. 45 X350

Photo 46.

The uterine muscle which is invaded by the carcinomatous growth shows plenty of fat. Sudan IV.

Case No. 2946/57.

Photo 47.

A non-stained section of the above case, examined with polarised light, showing doubly refractile crystals in a Maltese-Cross pattern.

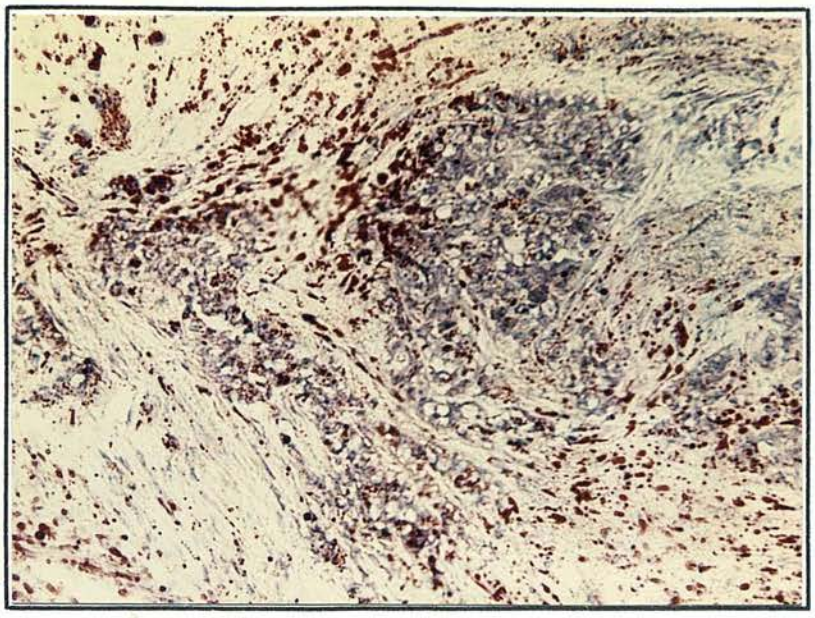


Fig. 46 X 165

Fig. 47 X 160

Photo 48.

Cancerous endometrium with one week radium before the operation. Showing black heaps of alkaline phosphatase activity while all cancer of the endometrium which had no radiation were negative for this enzyme except in the blood vessels. Compare with Photo 34.

Frozen section. Azo Method.

Case No. 3038/57.

Photo 49.

Acid phosphatase is at least not increased more than seen in the cases which had no radium.

Frozen section. Azo method. The same as previous case.

Fig. 48 X 125

Fig. 49 X 165

Photo 50.

Large amounts of mucin in the lumens of glands of cancerous endometrium irradiated for one week.

Southgate's Mucicarmine stain. The same as previous case.

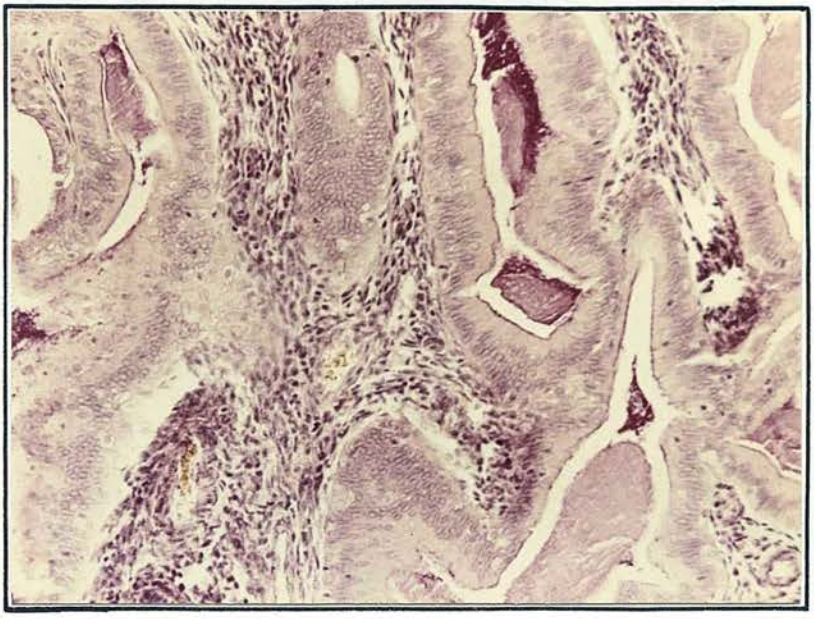


Fig. 50 X 165

PHOTOGRAPHS OF THE PLACENTA

1st Trimester

Photo 51.

Acid phosphatase in a normal 9 weeks' old placenta. Site: the trophoblast including Langhan's cells in some parts of the trophoblastic cover of villi. Paraffin section. Gomori Method.

Case No. 1222/57.

Photo 52.

Acid phosphatase in a normal 13 weeks' old placenta, present in all the syncytial covering of the chorionic villi. Frozen section - Azo-Coupling Method. Grenacher's Alum Carmine Counterstain.

Case No. 503/58.

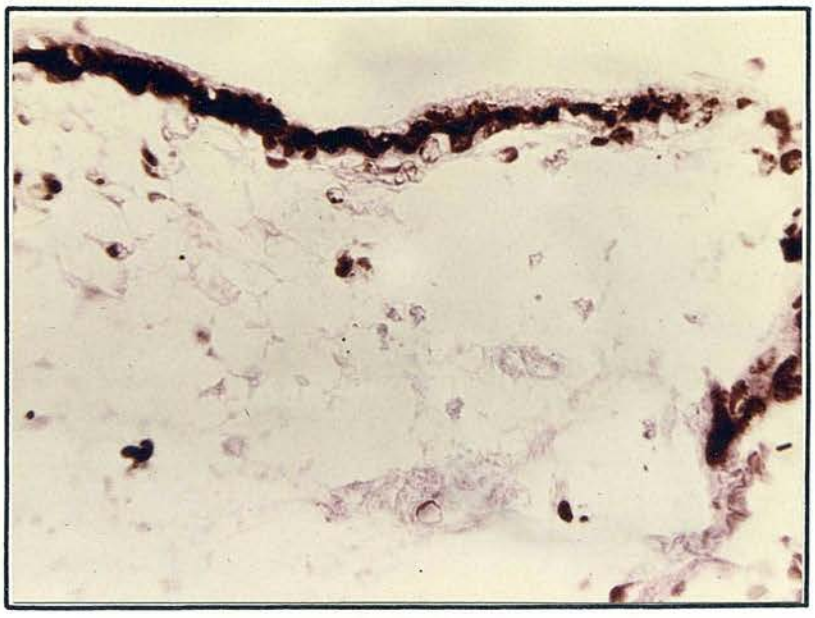


Fig. 51 X330

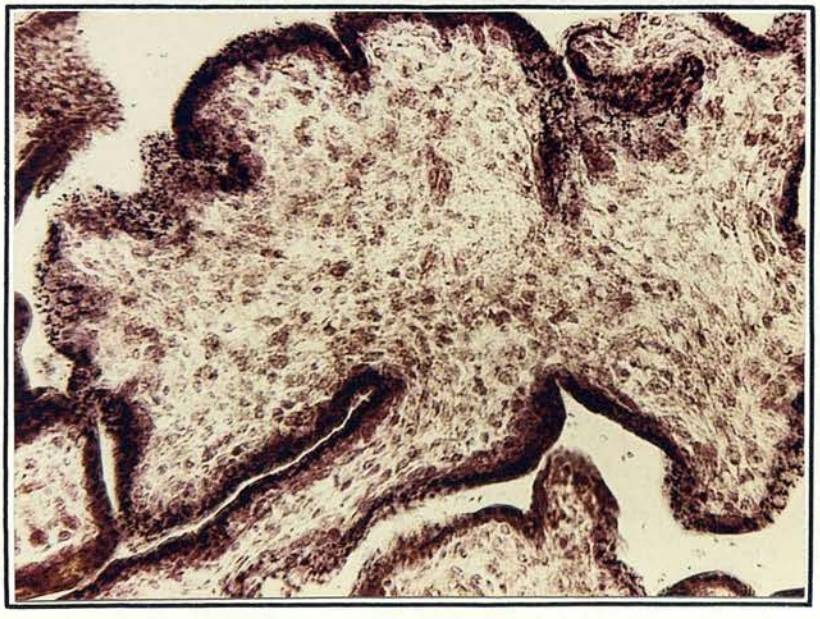


Fig. 52 X 145

Photo 53.

Alkaline phosphatase in a normal 13 weeks' old placenta seen in some parts of the syncytium, in the cytoplasm at the free border.

Frozen section. Azo-Coupling Method. No counter-stain.

Case No. 503/58.

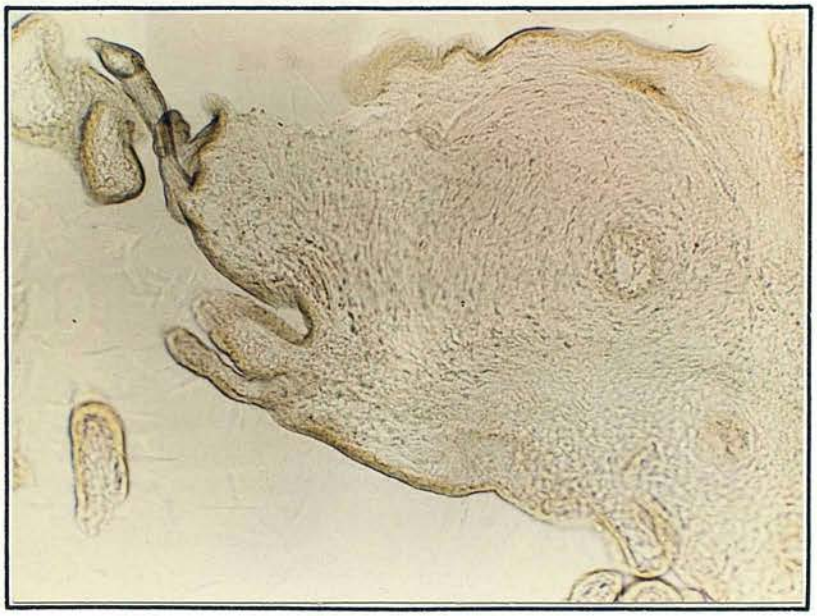


Fig. 53 X 90

Photo 54.

Non-specific esterase in a normal 13 weeks' old placenta. A strong colour reaction is present all over the syncytial cover of all the villi within the cytoplasm.

Frozen section. Azo-Coupling Method. No counterstain.

Case No. 503/58.

Photo 55.

Multitudes of fat (red) in the syncytium of chorionic villi of a normal 12 weeks' old placenta.

Frozen section. Sudan IV stain - dil. haematoxylin counterstain.

Fig. 54 X 145

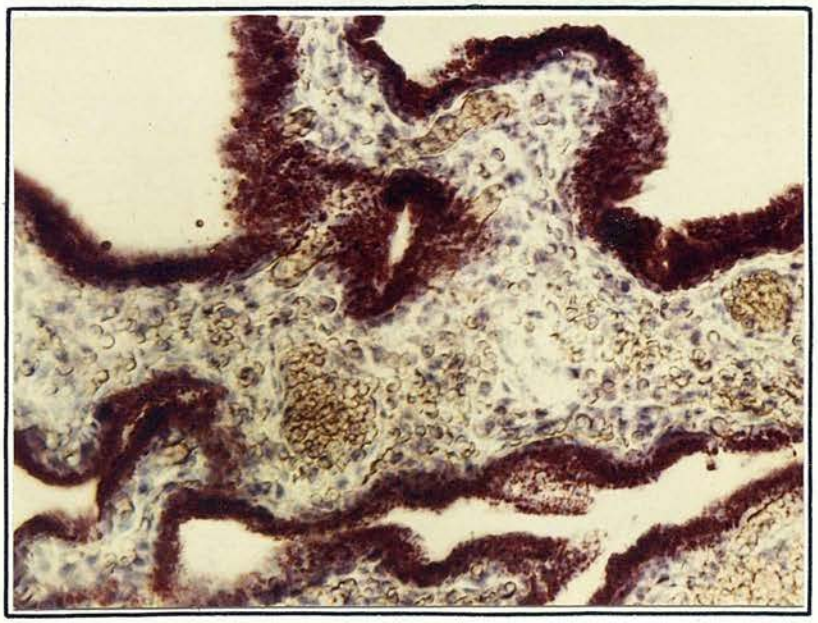


Fig. 55 X 220

Photo 56.

Fat is present in the syncytium but not in the Langhan's cells. Normal 3 months' old placenta.

Frozen section. Sudan IV stain - very diluted haematoxylin counterstain.

Case No. 3805/56.

Photo 57.

Fat in decidual cells in normal three months' pregnancy. Frozen section. Sudan IV stain.

This is the decidua of the above mentioned case.

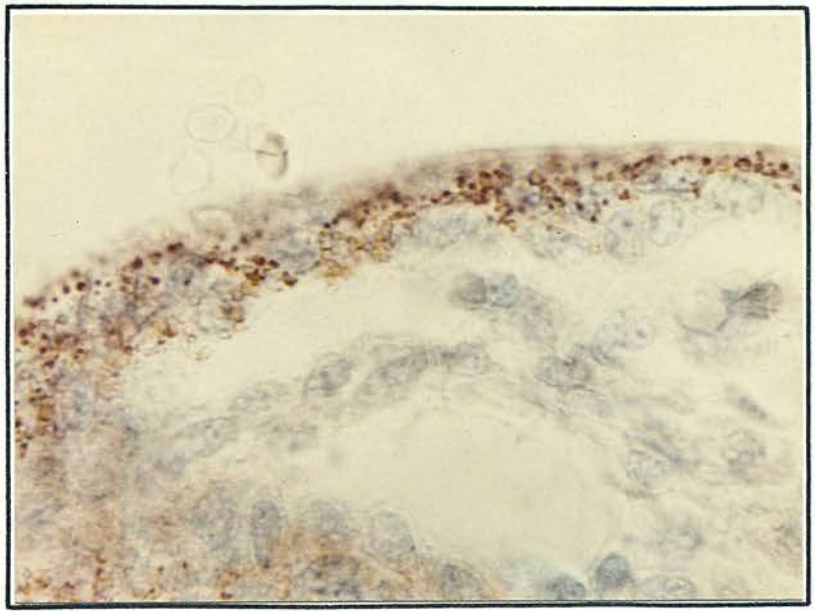


Fig. 56 X 745

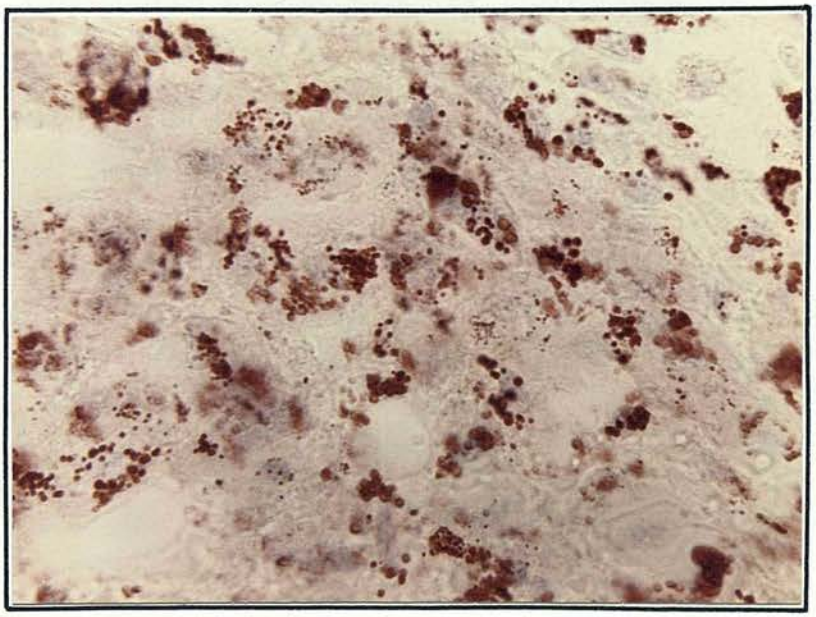


Fig. 57 X 350

Photo 58.

Doubly refractile fat in the decidua of 4 weeks' pregnancy.

Photo 59.

Glycogen in the cellular trophoblast and in the stroma of the newly formed villus.

Best's Carmine Stain. Case No. 503/58 - 13 weeks' normal placenta.

Fig. 58 X 120

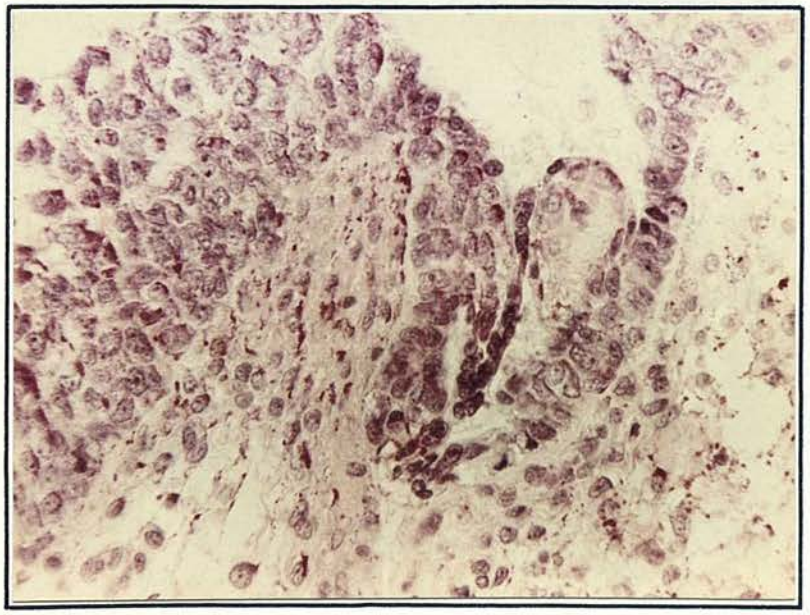


Fig. 59 X 350

Photo 60.

Cytoplasmic basophilia in the decidua of one month's pregnancy - in decidual cells and glandular epithelium. (This basophilia was abolished after treatment of the control slide with Ribonuclease.)

Eosin Methylene Blue Stain.

Case No. 569/57.

Photo 61.

A 9 weeks' normal placenta showing intense cytoplasmic basophilia in the syncytium of a chorionic villus (to the right). Cytoplasmic basophilia of lesser intensity is seen in Langhan's layer. The differentiating trophoblastic cells in the cytotrophoblastic cell column (to the left) exhibit a marked degree of basophilia.

Eosin Methylene Blue Stain.

Case No. 1222/57.

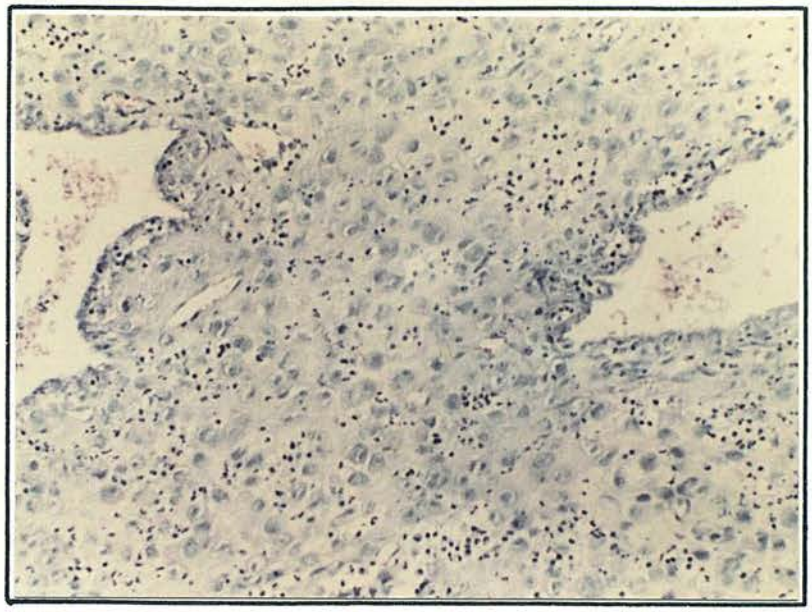


Fig. 60 X 125

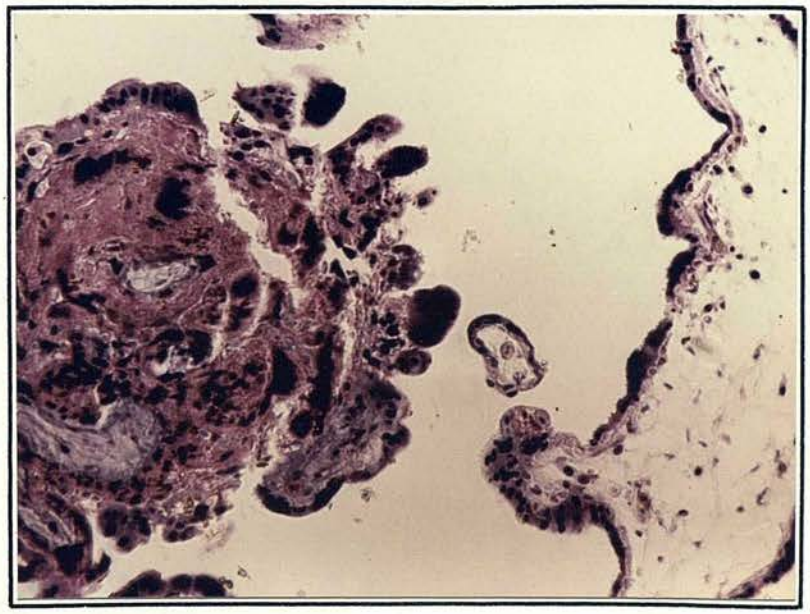


Fig. 61 X 160

2nd Trimester

Photo 62.

Alkaline phosphatase in the cytoplasm of all the syncytial covering of chorionic villi (black deposits). Frozen section. Azo-Coupling Method. A 20 weeks' normal placenta.

Case No. 5205/57.

Photo 63.

Acid phosphatase in the syncytium of all villi but the decidua is negative.

Frozen section. Azo-Coupling Method. A 20 weeks' normal placenta.

Case No. 5205/57.

Fig. 62 X 120

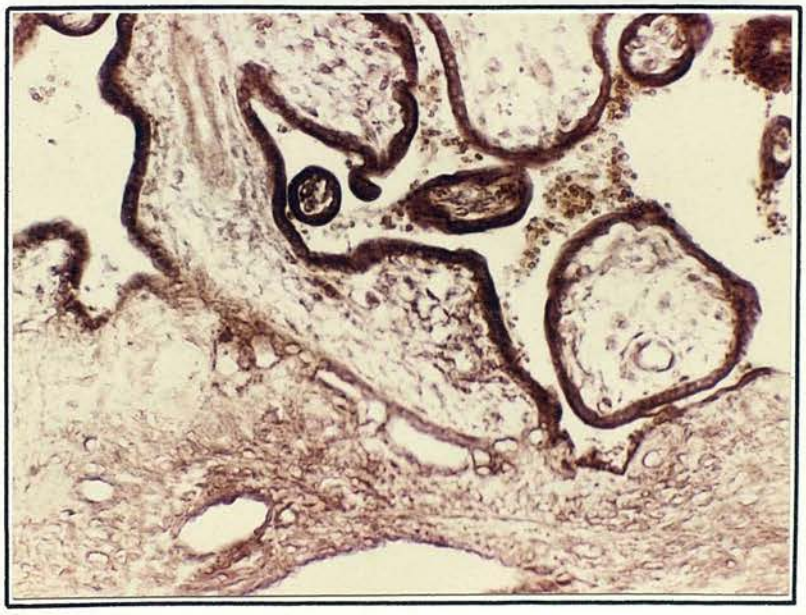


Fig. 63 X 120

Photo 64.

Non-specific esterase (as black deposits) in a normal twenty weeks' old placenta.

Sites:

1. In the cytoplasm of the syncytium.
2. In the stroma cells of some chorionic villi (as those seen in the figure).
3. In some cells, glands and blood vessels in the decidua.

Frozen section. Azo-Coupling Method.

Case No. 5205/57.

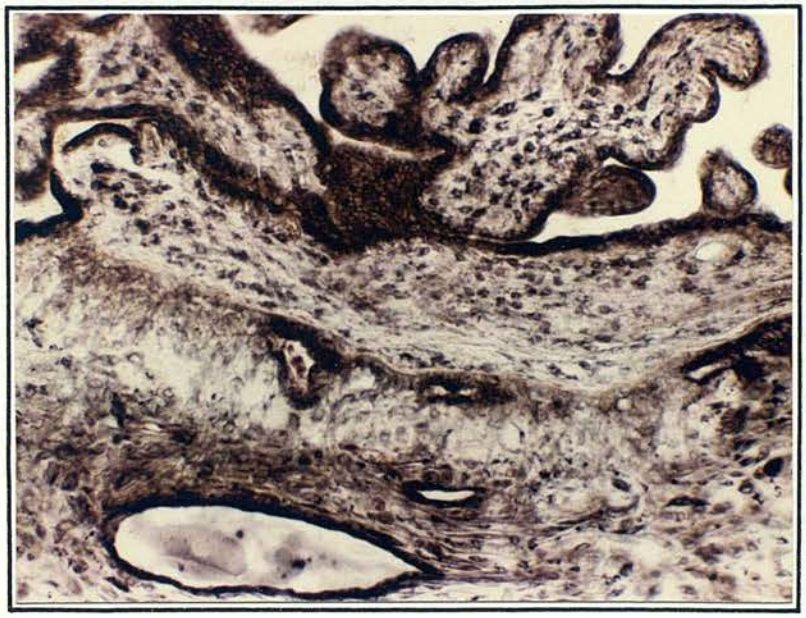


Fig. 64 X100

Photo 65.

Neutral fat in the stroma of a villus in a normal twenty weeks' old placenta. (The villi which showed fat in the stroma also showed non-specific esterase activity.)

Frozen section. Sudan IV stain.

Case No. 5205/57.

Photo 66.

Schultz test gave a green colour in the stroma of villi which showed fat. A twenty weeks' normal placenta.

Case No. 5205/57.

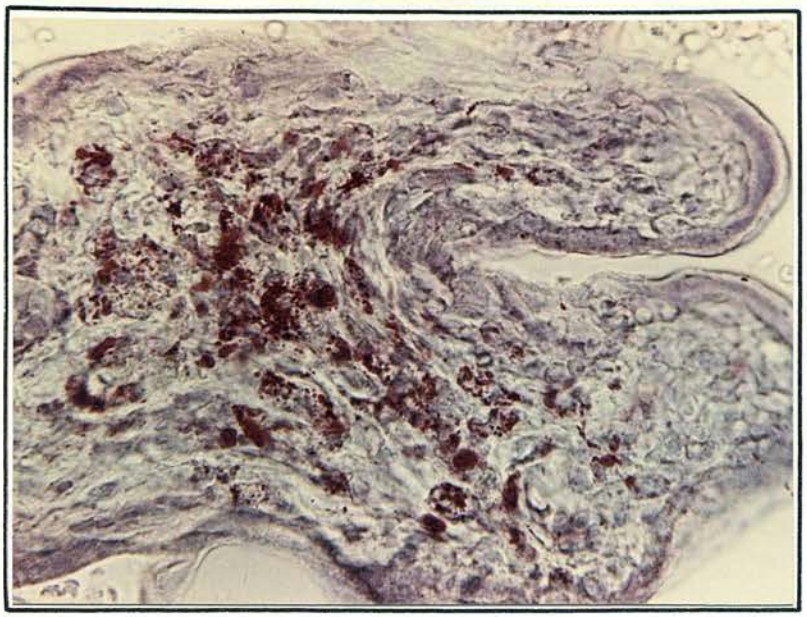


Fig. 65 X330

Fig. 66 X350

Photo 67.

Doubly refractile material in the lumen of a blood vessel in the decidua of a normal 20 weeks' old placenta. (This material gave a green colour with Schultz test and most probably is due to the cholestrol compounds normally present in the maternal blood.)

Fig. 67 X 150

Photo 68.

Glycogen (red) in decidual cells in a normal twenty weeks' old placenta - Best's Carmine Stain.

Case No. 5205/57.

Photo 69.

Calcium as fine black deposits in the stroma of a chorionic villus in a normal placenta 18 weeks' old.

Von Kossa Method.

Case No. 1259/57.

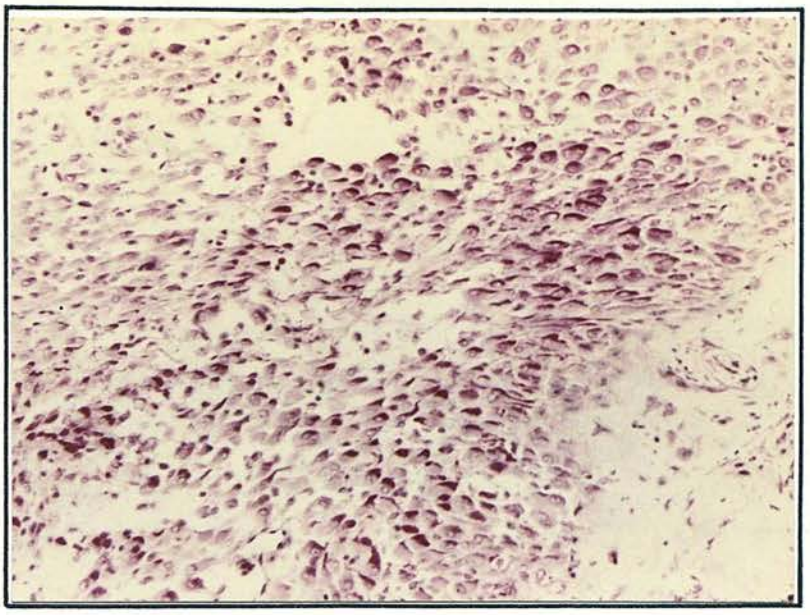


Fig. 68 X 140

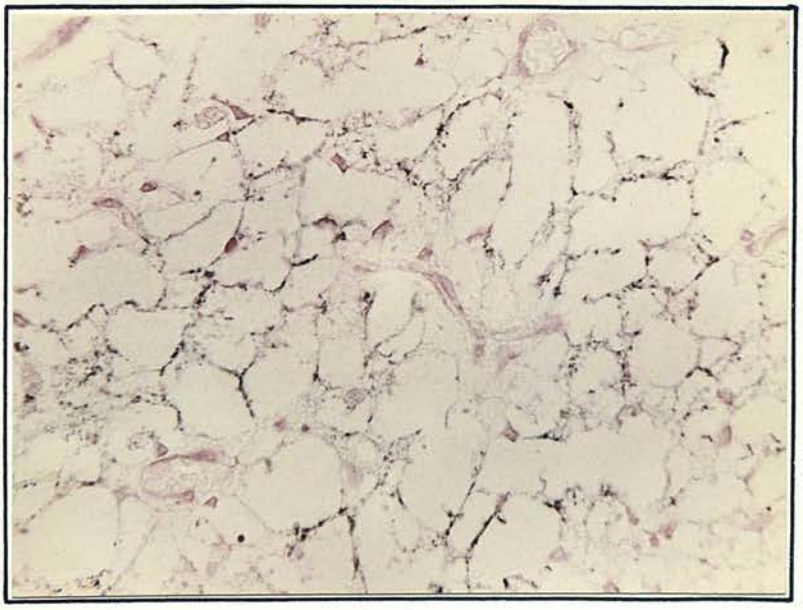


Fig. 69 X 350

3rd Trimester

Photo 70.

H.E.: 28 weeks' normal placenta. In the photo many new villi are sprouting.

Case No. 4990/57.

Photo 71.

H.E.: 35 weeks' normal placenta. New villi are still developing.

Case No. 3125/57.

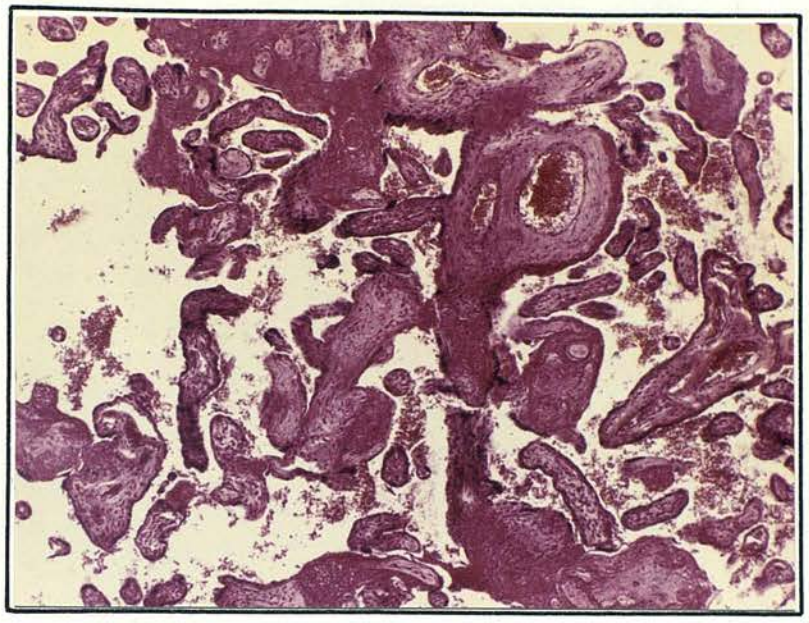


Fig. 70 X50

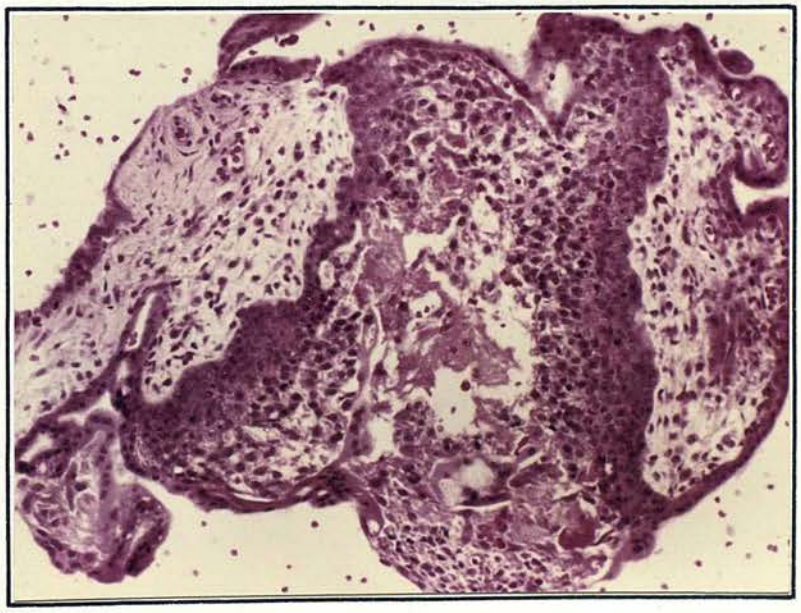


Fig. 71 X 130

Photo 72.

H.E.: Normal placenta 30 weeks' old. Variation in size and texture of villi.

Case No. 920/57.

Photo 73.

H.E.: A 34 weeks' normal placenta when Tenney's nodes start to show.

Case No. 517/57.

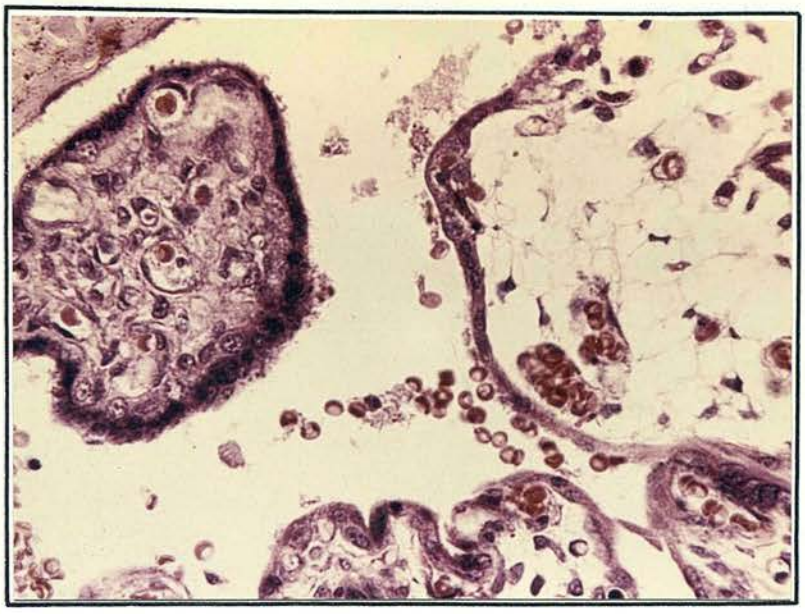


Fig. 72 X 350

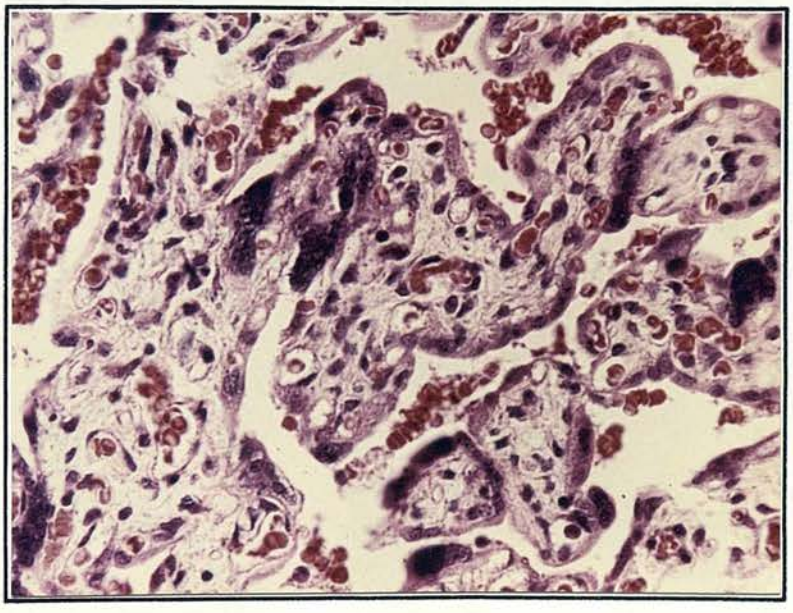


Fig. 73 X 350

Photo 74.

Alkaline phosphatase in 28 weeks' normal placenta.

Site: Syncytium of all villi (compare with younger placentas).

Frozen section. Azo-Coupling Method.

Case No. 4990/57.

Photo 75.

Acid phosphatase in 28 weeks' normal placenta.

Site: Syncytium of all villi.

Frozen section. Azo-Coupling Method.

Case No. 4990/57.

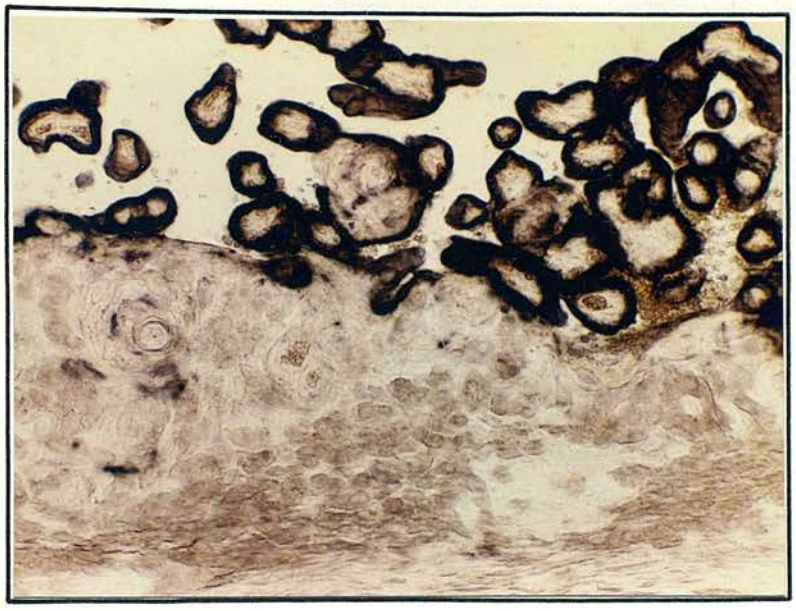


Fig. 74 X100

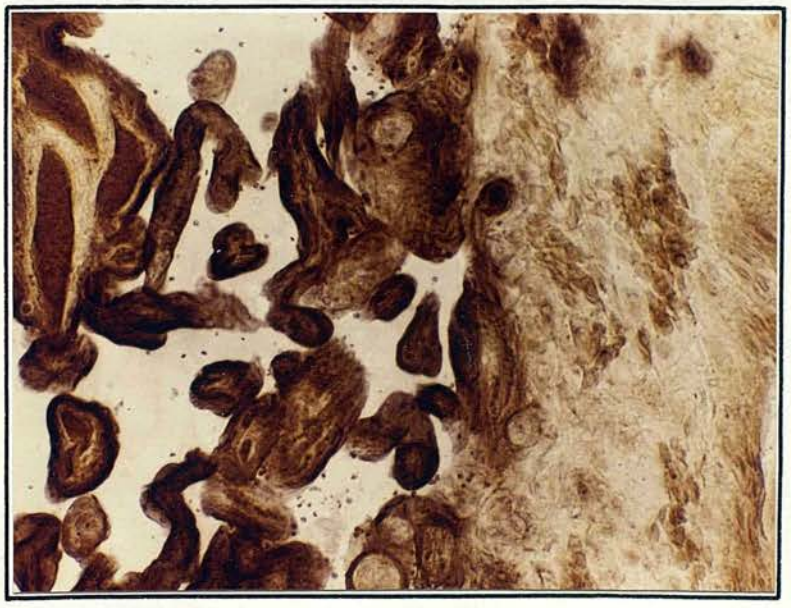


Fig. 75 X100

Photo 76.

·Non-specific esterase in some decidual cells in a normal 8 weeks' placenta.

Frozen section. Azo-Coupling Method.

Case No.4990/57.

Photo 77.

Glycogen as fine red granules in the stroma of a stem villus - 8 weeks' normal placenta.

Best's Carmine Stain.

Case No. 4990/57.

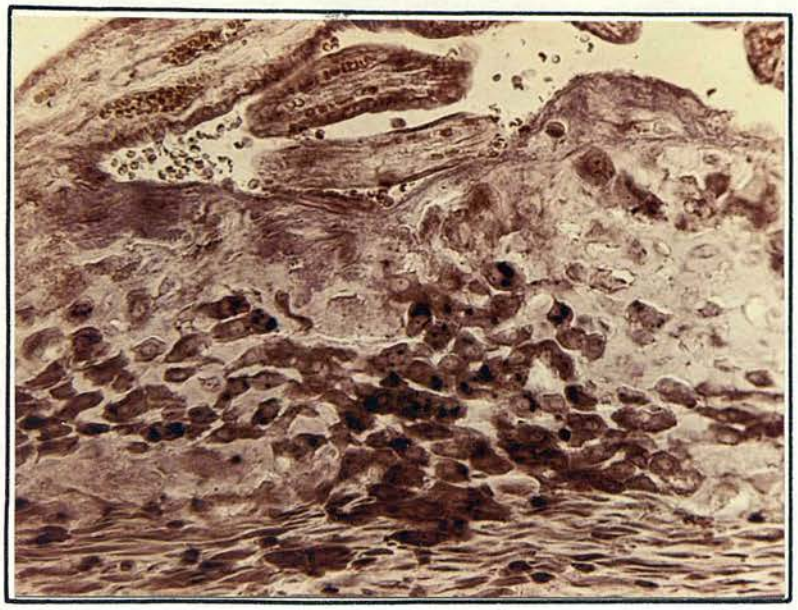


Fig. 76 X160

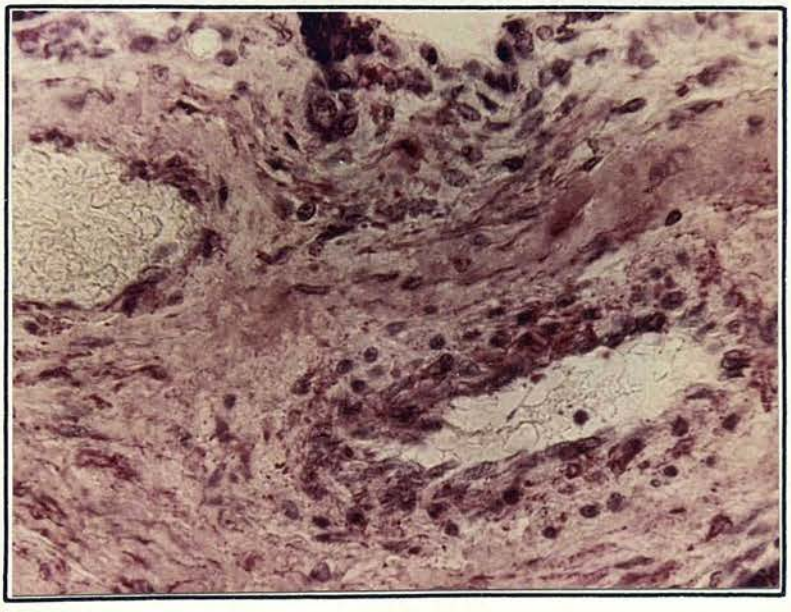


Fig. 77 X 350

NORMAL PLACENTA AT TERM

Photo 78.

Calcium deposits (brown) between villi in a normal placenta at term. Von Kossa stain.

Case No. 3038/57.

Photo 79.

Alkaline phosphatase (black) normal placenta at term.

Site: Syncytium of all chorionic villi - the patchy slight deposits in the decidua are in necrotising cells in the decidua.

Frozen section. Azo-Coupling Method.

Case No. 2551/57.

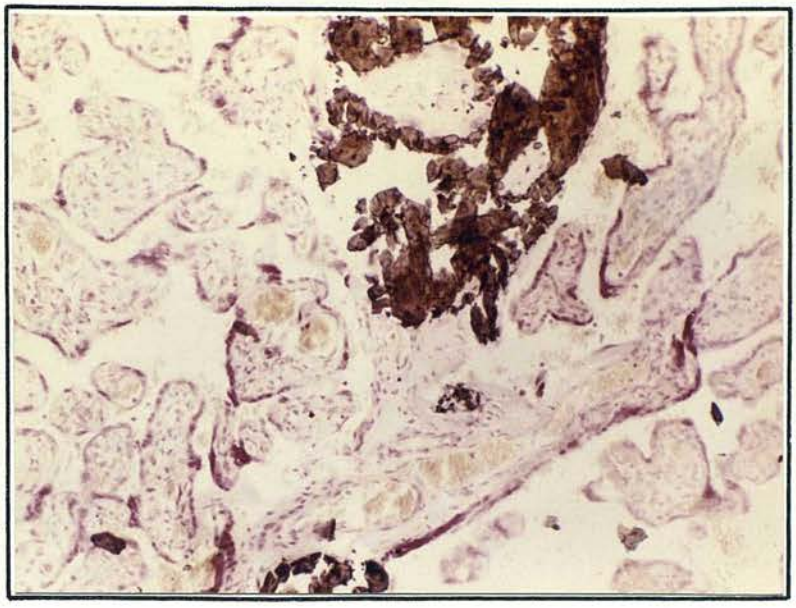


Fig. 78 X 115

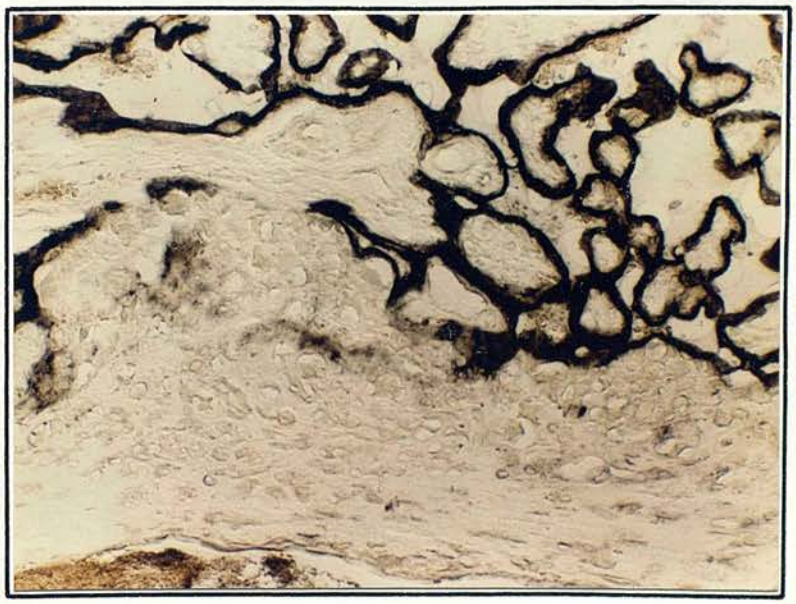


Fig. 79 X 110

Photo 80.

Acid phosphatase (black) in the syncytium of all chorionic villi in a normal placenta at term.

Frozen section. Azo-Coupling Method.

Case No. 2551/57.

Photo 81.

Acid phosphatase in a normal placenta at term by the paraffin method of Gomori - pH 4.8 showing a heavy nuclear reaction in the syncytium and in the stroma of a villus. Necrotising cells in the decidua also show the reaction.

Case No. 5783/56.

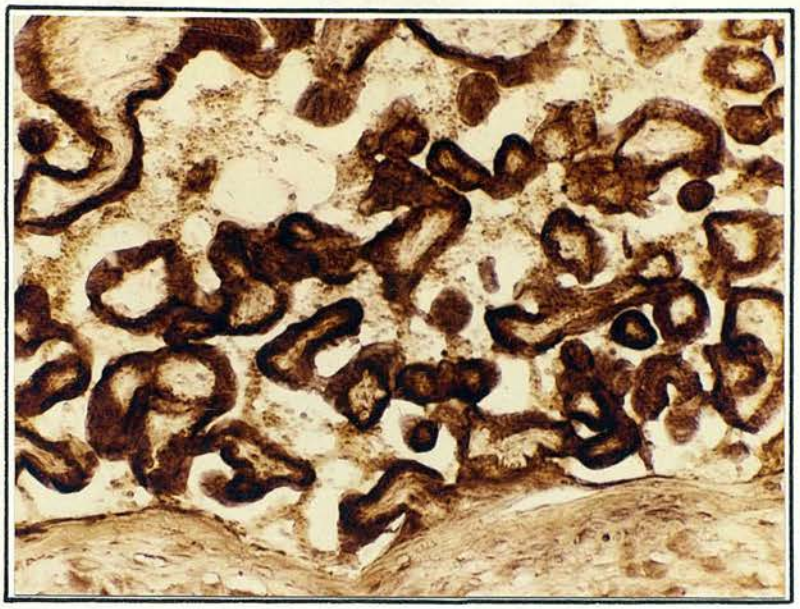


Fig. 80 X 110

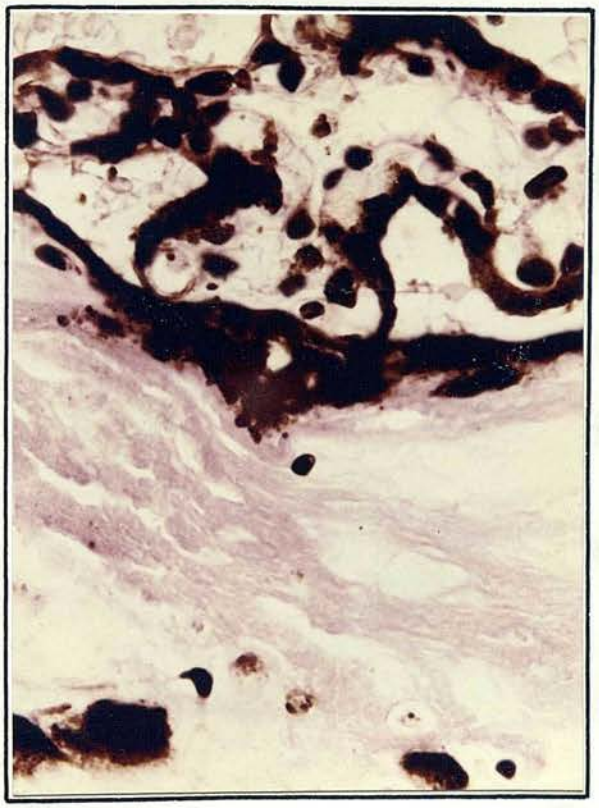


Fig. 81 X 465

Photo 82.

Glycogen granules (red) in the cytoplasm of decidual cells in a normal placenta at term.

Best's Carmine Stain.

Case No. 3038/57.

Photo 83.

A faint cytoplasmic basophilia in the syncytium in a normal placenta at term.

Eosin Methylene Blue Stain.

Case No. 505/57.

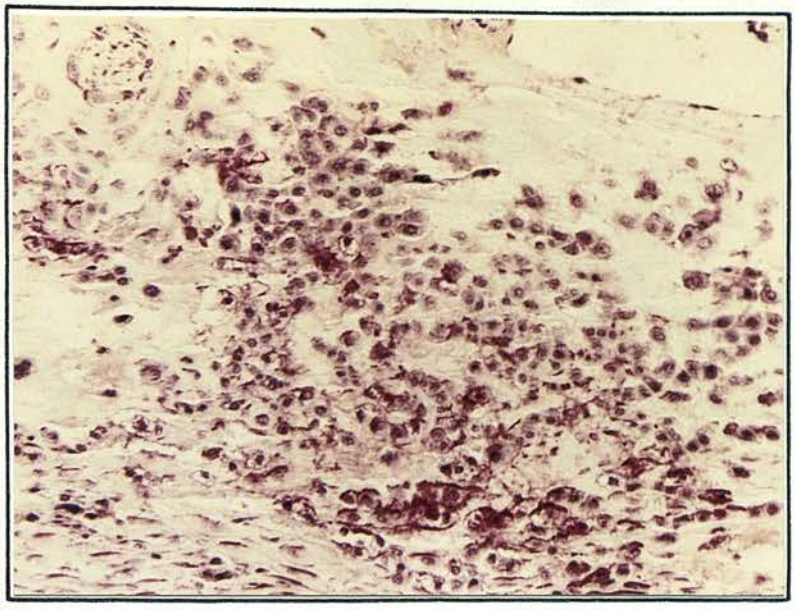


Fig. 82 X 150

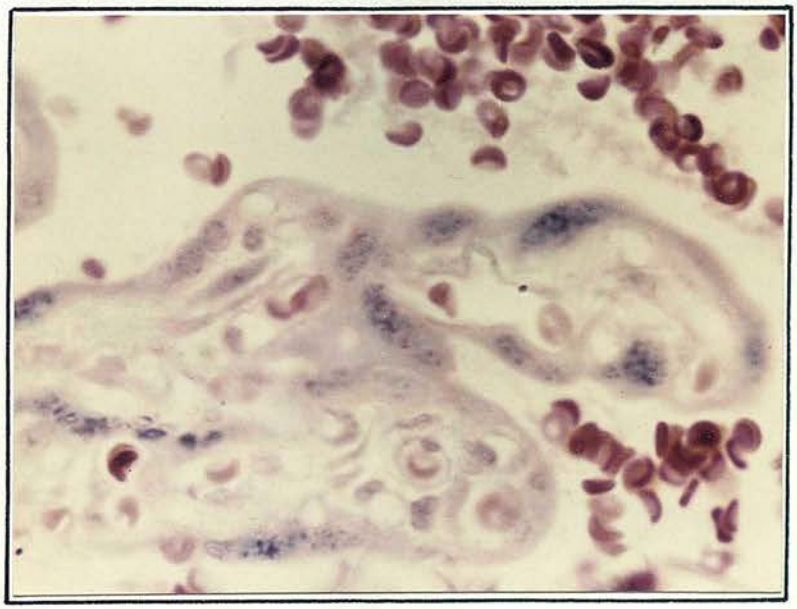


Fig. 83 X 745

Photo 84.

Non-specific esterase (black) in some decidual cells in a normal placenta at term.

Frozen section. Coupling-Azo Method.

Case No. 5012/57.

Photo 85.

Sudanophilic fat granules (to the right) in some decidual cells in a normal placenta at term.

Case No. 185/58.

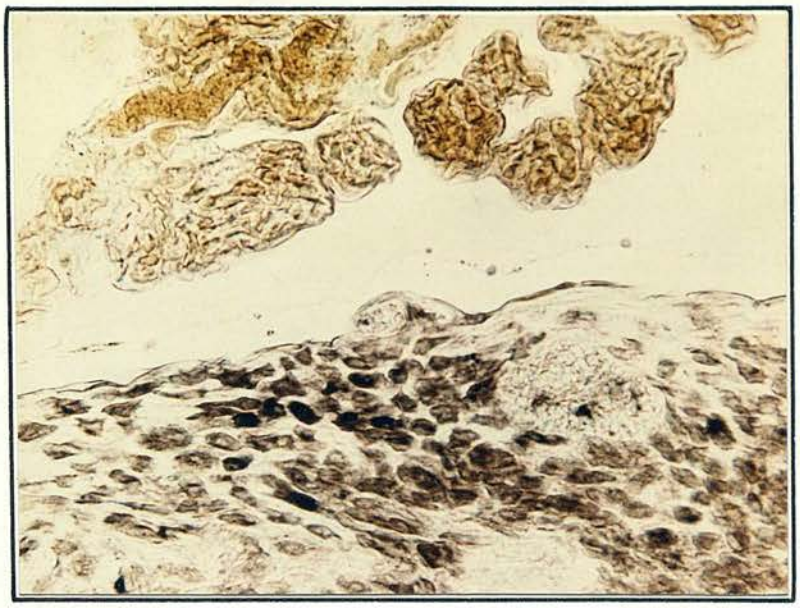


Fig. 84 X 165

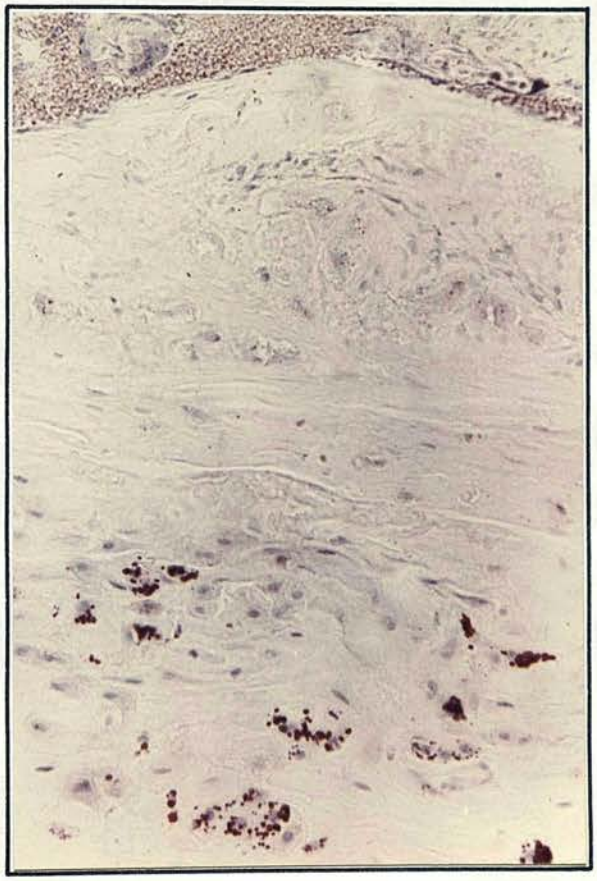


Fig. 85 X 150

Photo 86.

Fat in the decidua of the previous case under polarised light with high magnification. Formalin fixed frozen section. No stain.

Photo 87.

Schultz test in the same previous case. The fat in decidual cells gave a blue-green colour with Schultz test. The green colour is beginning to change into brown and the background from fawn to pink at the moment when the photograph was taken.

Fig. 86 X 135

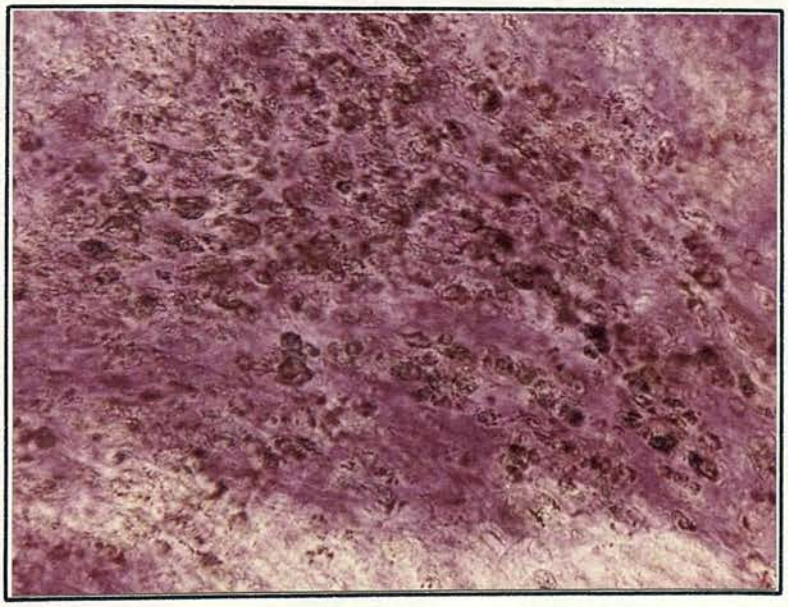


Fig. 87 X 175

Photo 88.

H.E.: Post-mature placenta showing extensive hyalinisation in the chorionic villi.

Case No. 296/57.

Photo 89.

Calcium deposits (increased) in post-mature placenta. Von Kőssa.

Case No.2865/57.

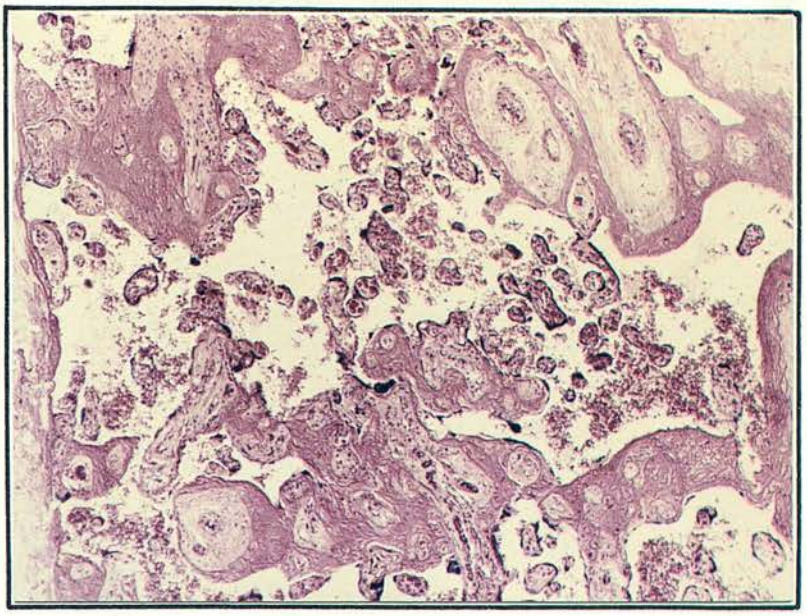


Fig. 88 X 50

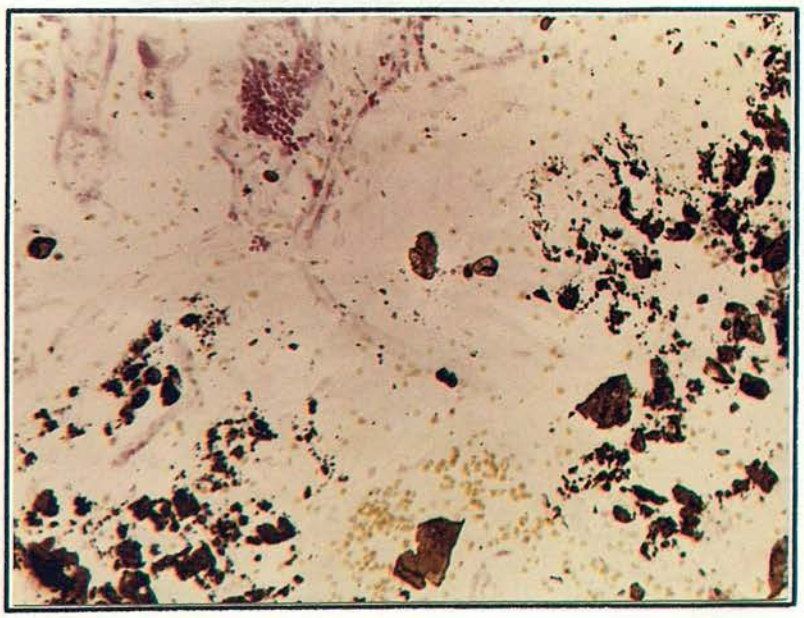


Fig. 89 X 165

Photo 92.

Non-specific esterase which was absent from the syncytium in normal placentae at term is now seen in the post-mature placenta. In the figure a few villi are seen amalgamated together with pinkish calcium deposits in the degenerating areas where remains of the colour reaction are present.

Frozen section. Azo-Coupling Method. Grenacher's Carmine Counterstain.

Case No. 5496/57.

Photo 93.

Sudanophilic fat in the post-mature placenta is only evident in the degenerating villi. In the figure some fat droplets (red) are seen in the degenerating villi which are adjacent to the decidua. The homogeneous violet patches are the sites of calcium deposition. The decidua shows no fat.

Case No. 5310/57.

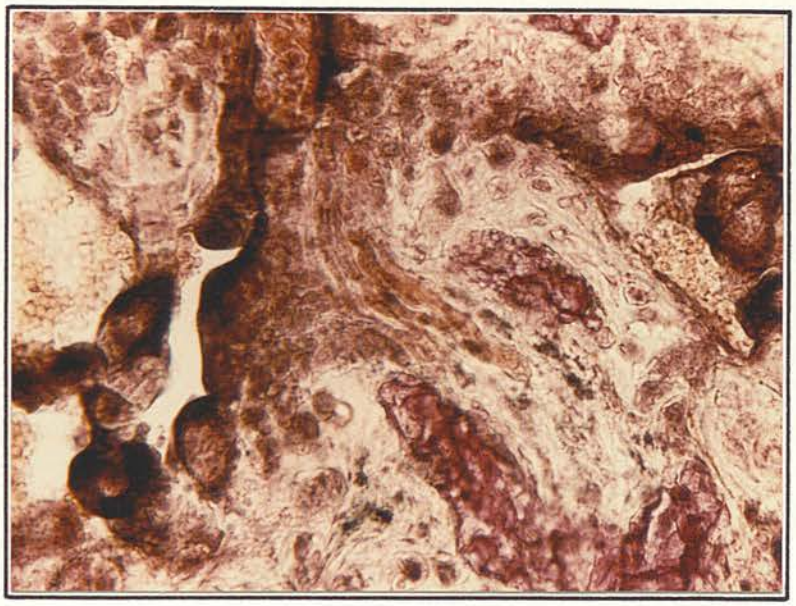


Fig. 92 X 165

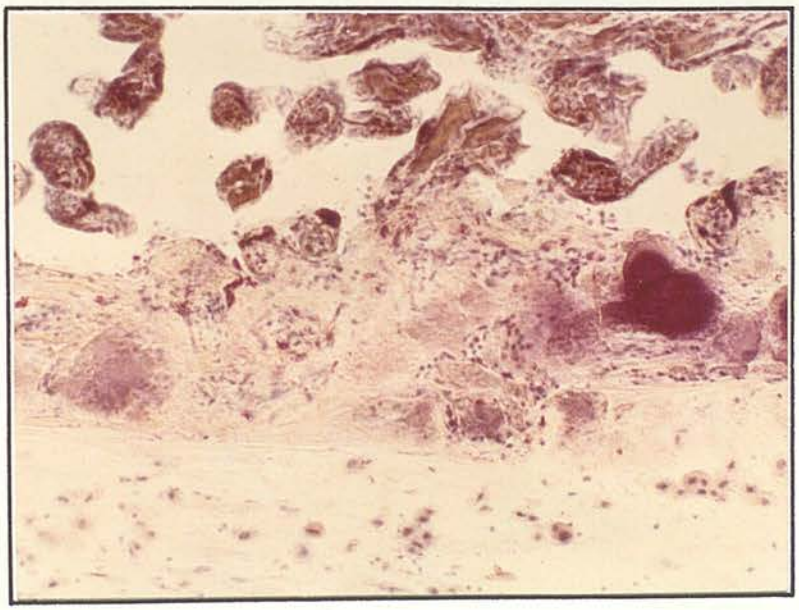


Fig. 93 X110

THE TOXAEMIC PLACENTA

Photo 94.

H.E.: 30 weeks' eclamptic placenta. Extensive hyalinisation in villi which are seen matted together. The decidual edge of the placenta (to the right) also shows hyalinisation.

Case No. 809/57.

Photo 95.

Calcium deposits in a 30 weeks' eclamptic placenta. Von Kóssa.

Case No. 809/57.

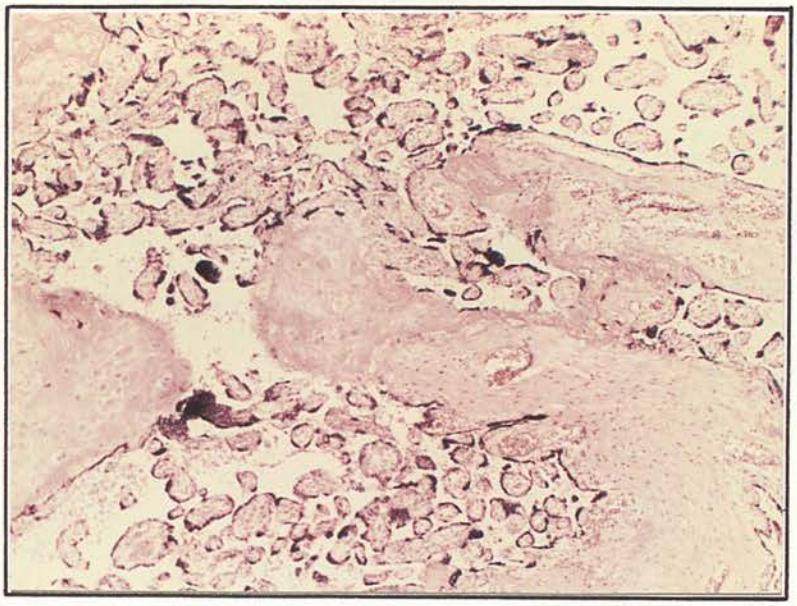


Fig. 94 X 50

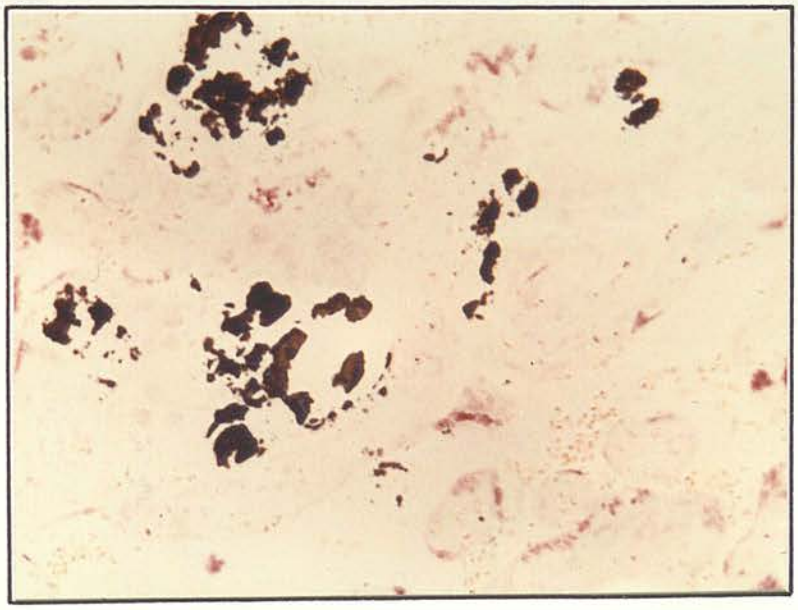


Fig. 95 X 165

Photo 96.

Alkaline phosphatase in a toxaemic placenta at term. (Compare with normal placenta at term.)

Frozen section. Azo-Coupling Method.

Case No. 5652/57.

Photo 97.

Acid phosphatase in a toxaemic placenta at term.

Frozen section. Azo-Coupling Method.

Case No. 5712/57.

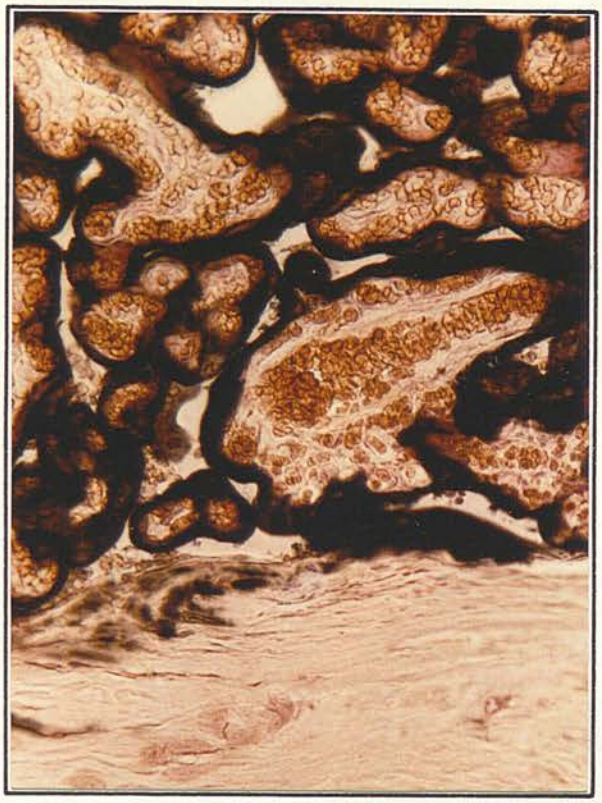


Fig. 96 X 165

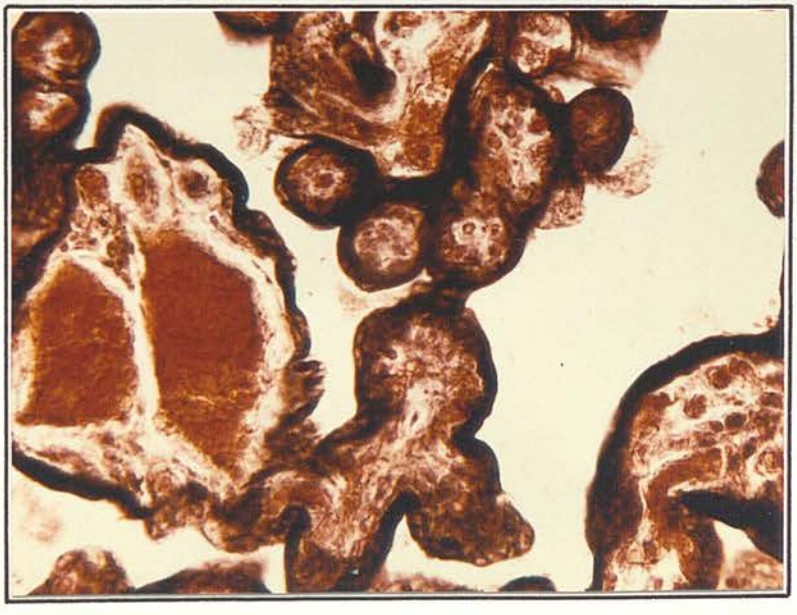


Fig. 97 X165

Photo 98.

Non-specific esterase in a toxaemic placenta at term. Compare with normal placenta at term where none of this enzymatic reaction is present in the syncytium.

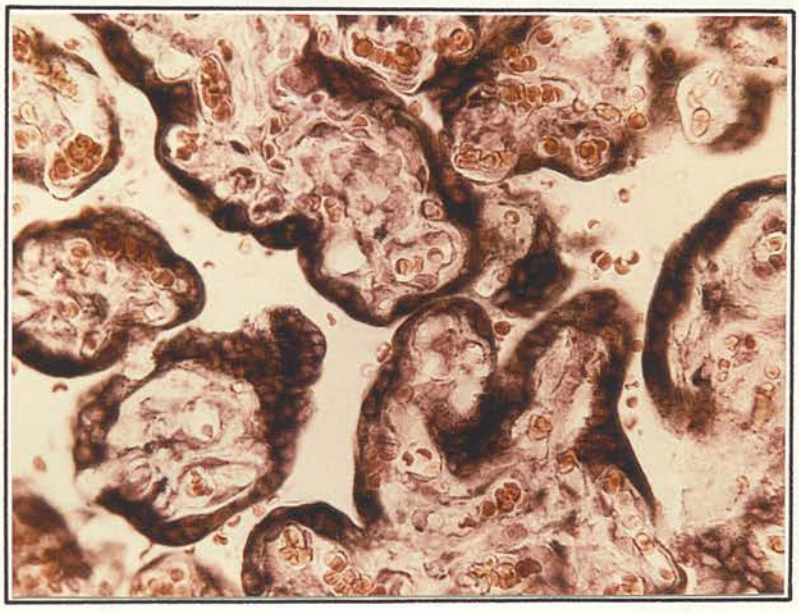


Fig. 98 X300

Photo 99.

30 weeks' eclamptic placenta where cytoplasmic basophilia is less than normal - compare with the following figure.

Case No. 809/57.

Photo 100.

Cytoplasmic basophilia in a normal 30 weeks' placenta. Eosin Methylene Blue Stain.

Case No. 459/57.

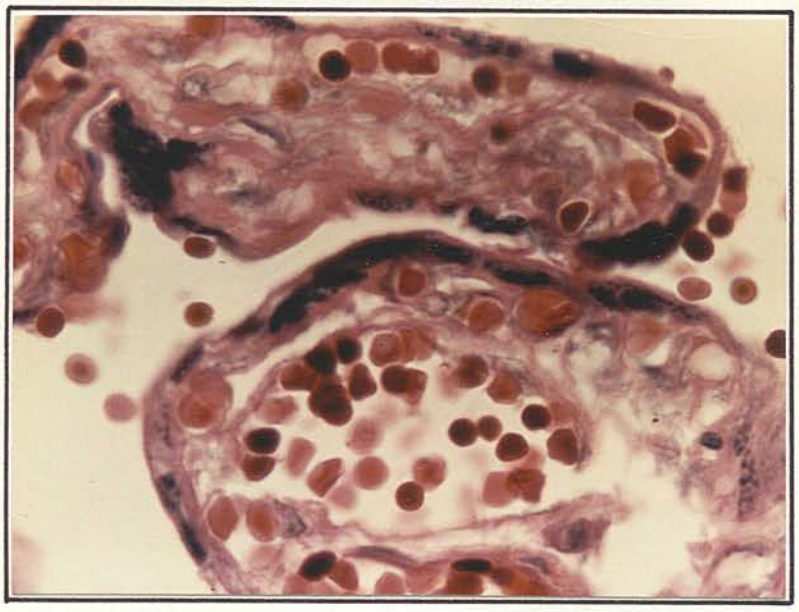


Fig. 99 X 745

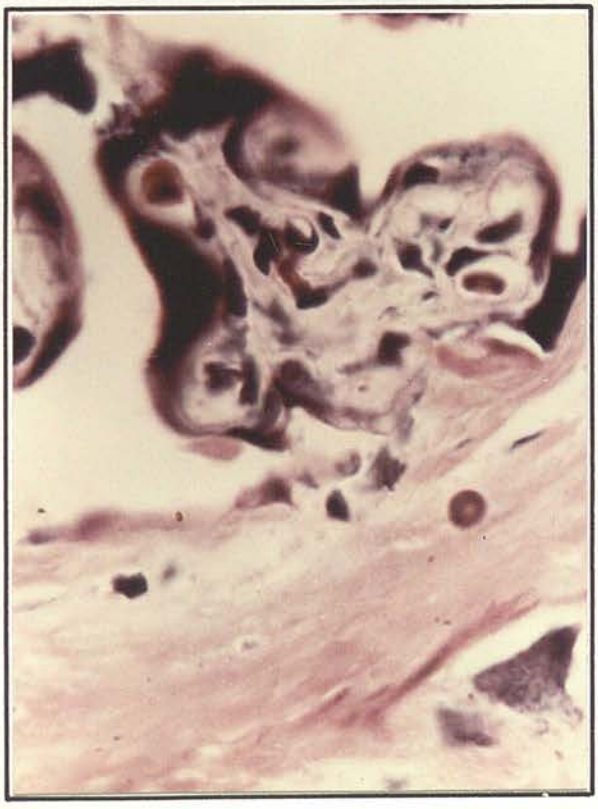


Fig. 100 X 745

Photo 101.

Doubly refractile layer of fat in the decidua in 38 weeks' toxaemic placenta.

Case No. 5781/57.

Photo 102.

The same as preceding case showing Sudanophilic fat in the decidua (to the left) and in degenerating villi (to the right).

Fig. 101 X 90

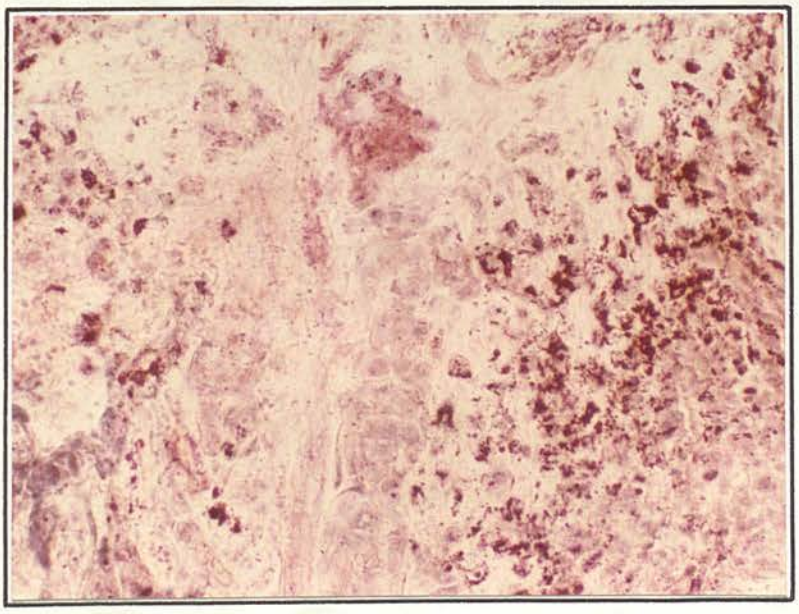


Fig. 102 X 110

Photo 103.

Fat in a degenerating strand of decidua - 37 weeks' - toxaemic placenta. Sudan IV.

Case No. 1205/58.

Photo 104.

Fatty degeneration in the walls of a blood vessel in the decidua in a case of toxaemia at term. Sudan IV.

Case No. 5712/57.

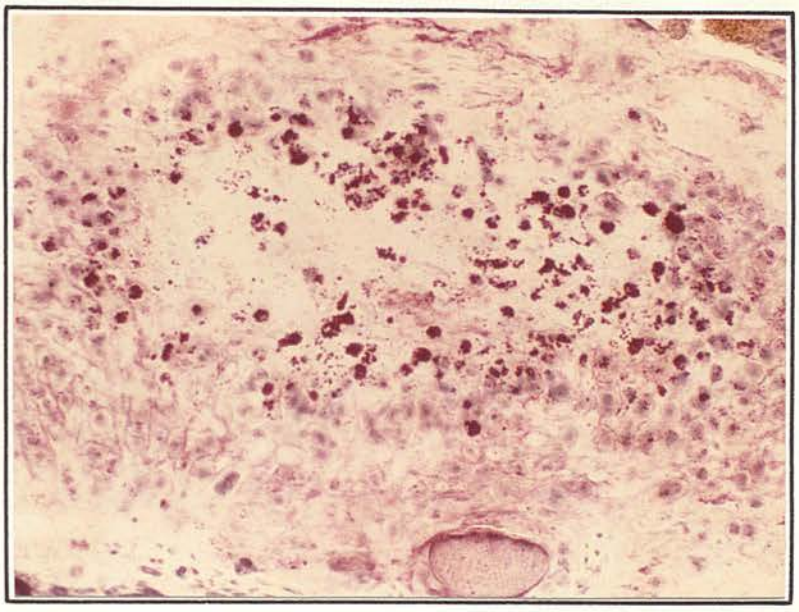


Fig. 103 X 110

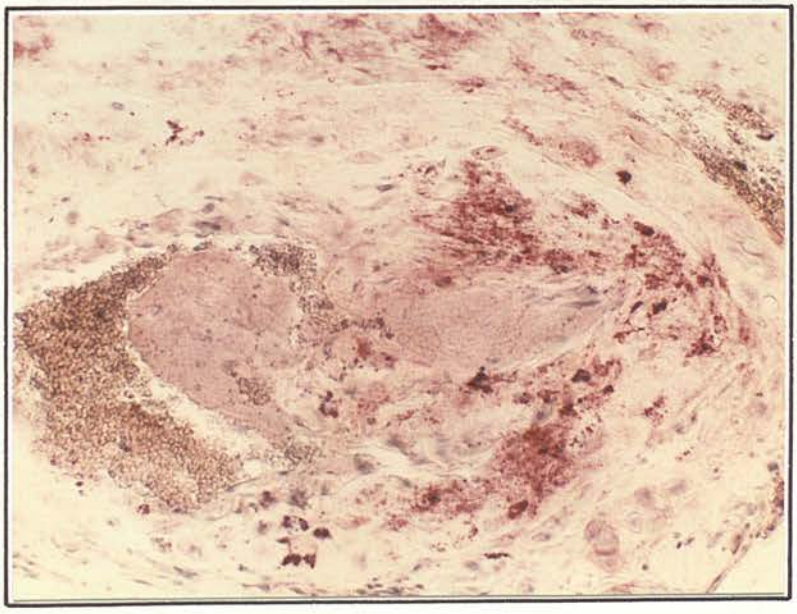


Fig. 104 X 110

Photo 105.

Toxaemia at term. Fatty degeneration in the secondary chorionic villi adjacent to a stem villus. Sudan IV.

Case No. 5529/57.

Photo 106.

Fatty degeneration at the border of an infarction in a 38 weeks' toxaemic placenta. The inside of the infarction (to the left) has already degenerated.

The relatively healthier villi (to the right) are also suffering but to a lesser extent.

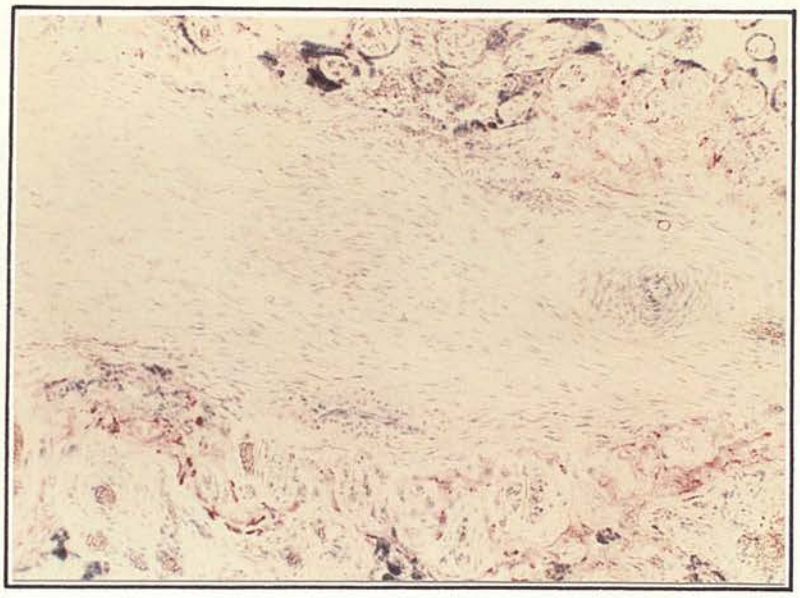


Fig. 105 X 85

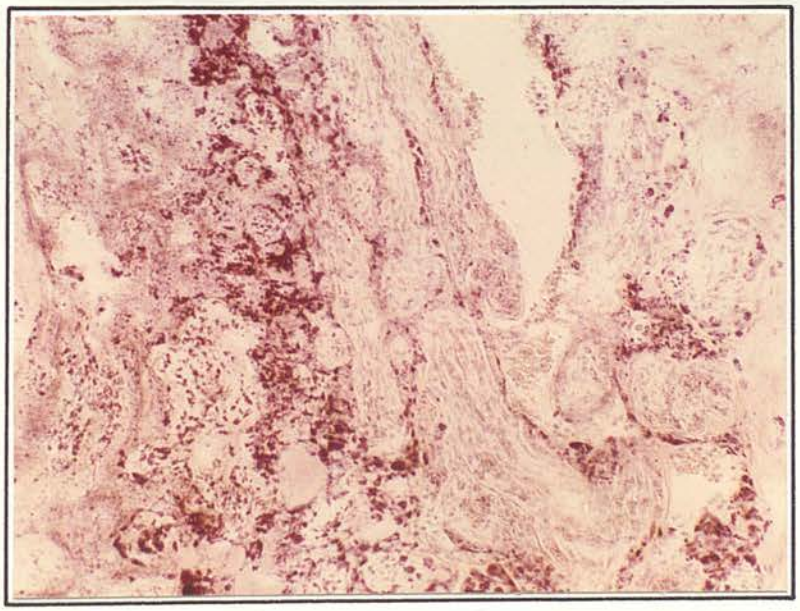


Fig. 106 X 85

PLACENTA IN OTHER PATHOLOGICAL CONDITIONS

Photo 107.

Fat in the decidua in a case of Type II Nephritis at 37 weeks' pregnancy. Sudan IV.

Case No. 5311/57.

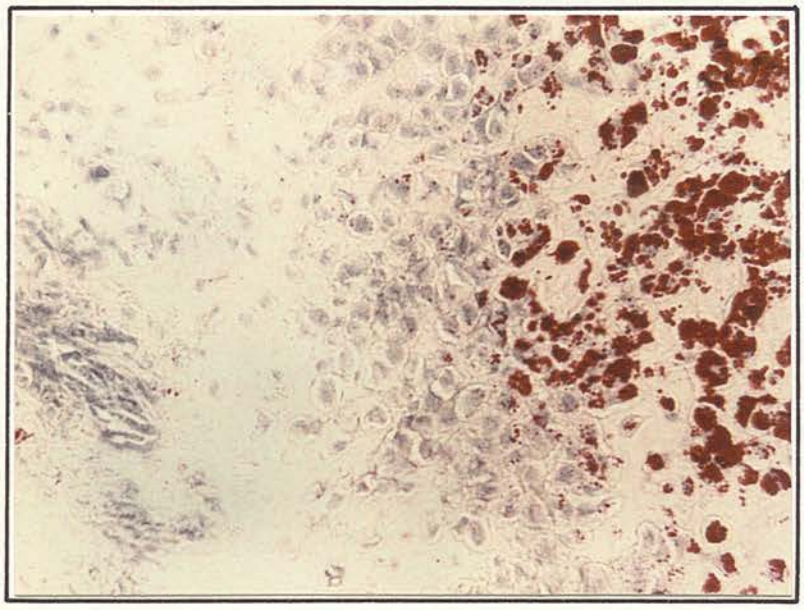


Fig. 107 X 145

Photo 108.

Non-specific esterase in a 36 weeks' placenta from a patient with diabetes mellitus.

Sites: in decidual cells, inside a decidual blood vessel (upper left corner) in the stroma of some villi (which showed fat) and in the syncytium.

Frozen section. Azo-Coupling Method.

Case No. 5326/57.

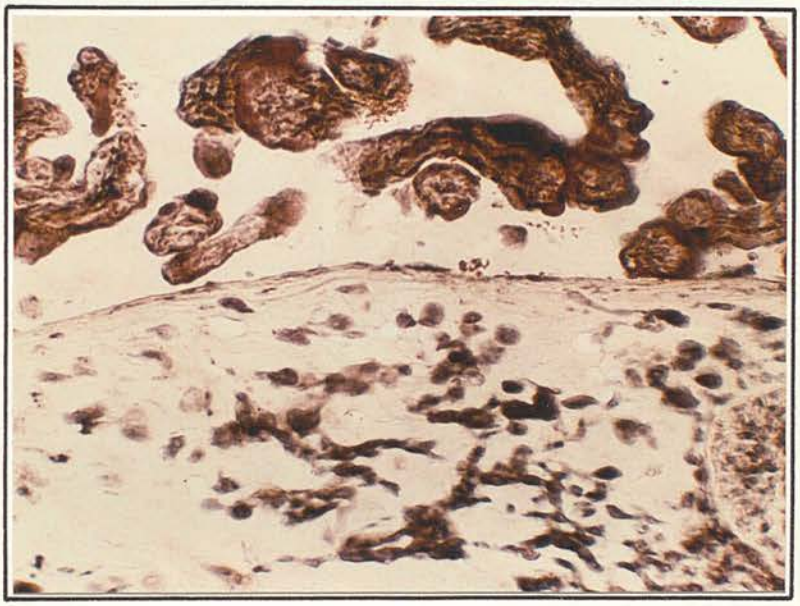


Fig. 108 X 125

Photo 109.

Fat in the stroma of healthy chorionic villi in 36 weeks' placenta from the same previous case of diabetes mellitus.

Photo 110.

The syncytium of a 12 weeks' placenta from a patient with severe diabetes mellitus. The syncytial fat is less than in a normal placenta of the same age. Compare with Figure 55. Sudan IV.

Case No. 5751/57.

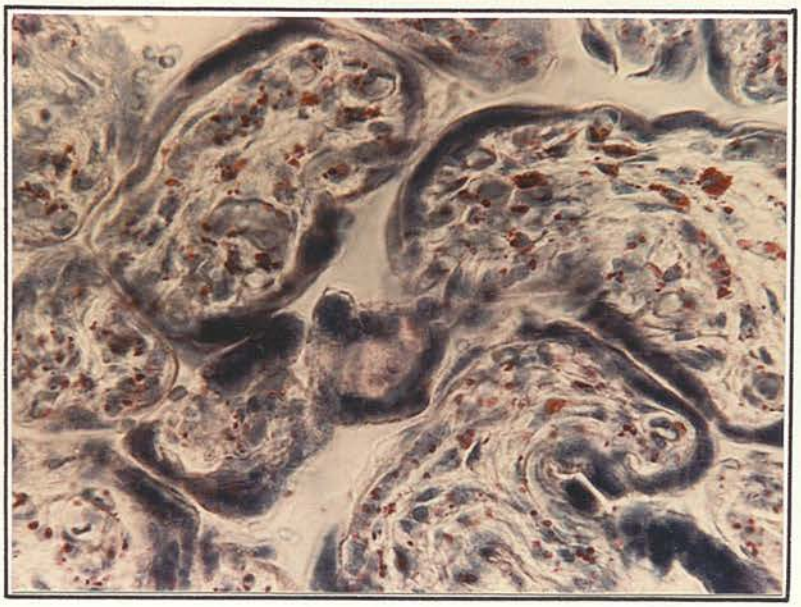


Fig. 109 X350

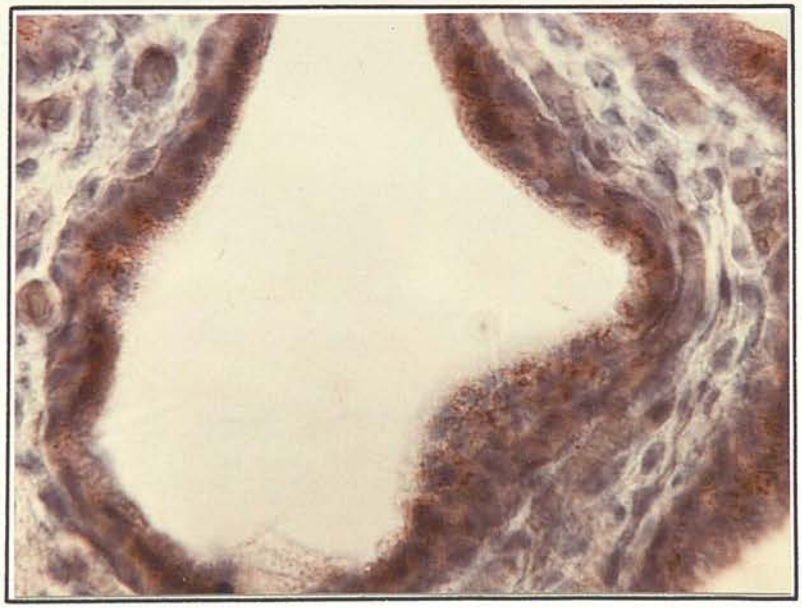


Fig. 110 X 430

Photo 111.

Eosin Methylene Blue stained section from a normal 24 weeks' placenta.

Case No. 555/57.

Photo 112.

Eosin Methylene Blue stained section from a 34 weeks' placenta of a patient suffering from diabetes mellitus.

Unlike toxaemia, the cytoplasmic basophilia is not diminished.

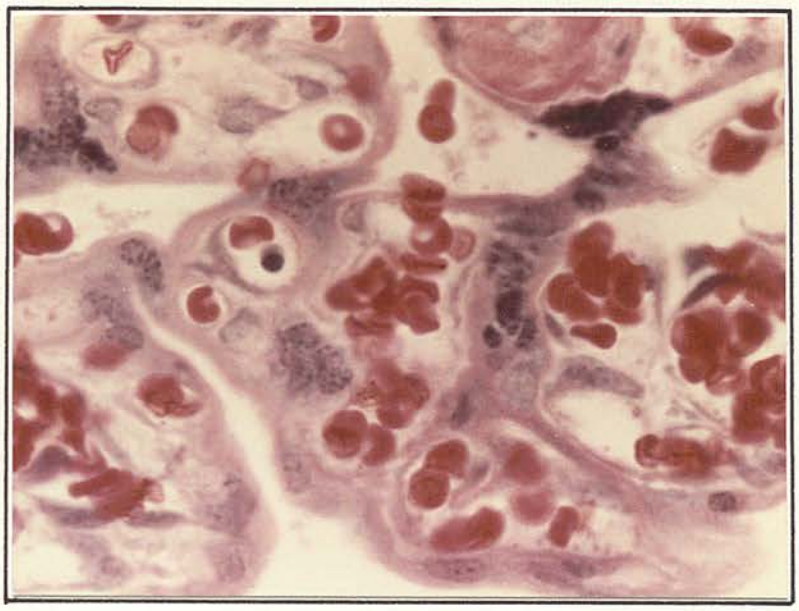


Fig. 111 X 745

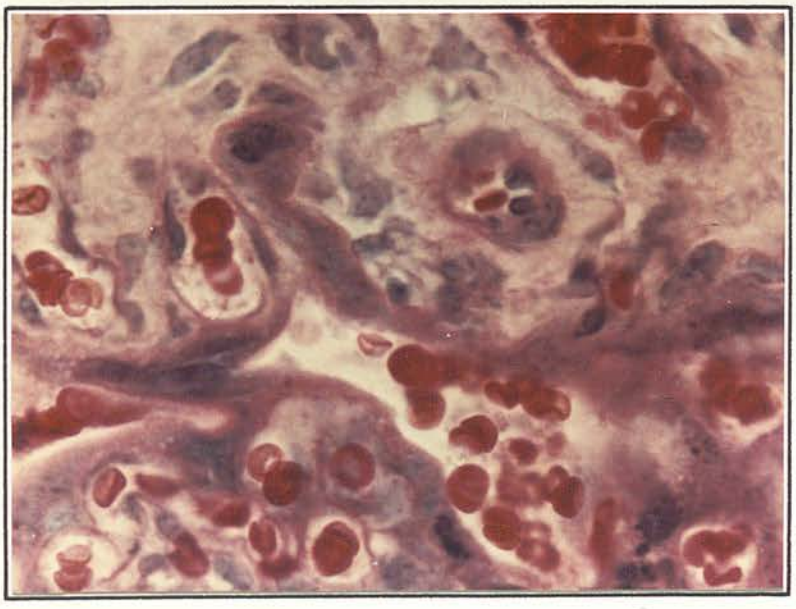


Fig. 112 X745

Photo 113.

Alkaline phosphatase in trophoblastic cells in vesicular mole. Paraffin Calcium-Cobalt Method.

Case No. 999/57.

Photo 114.

Acid phosphatase in decidual cells (in the nuclei). Vesicular Mole. Paraffin Gomori Method.

Case No. 1634/57.

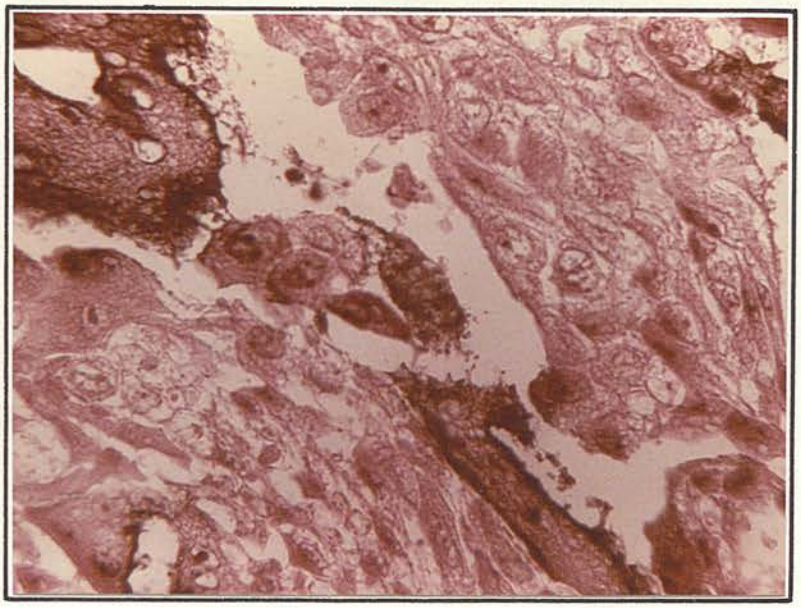


Fig. 113 X 350

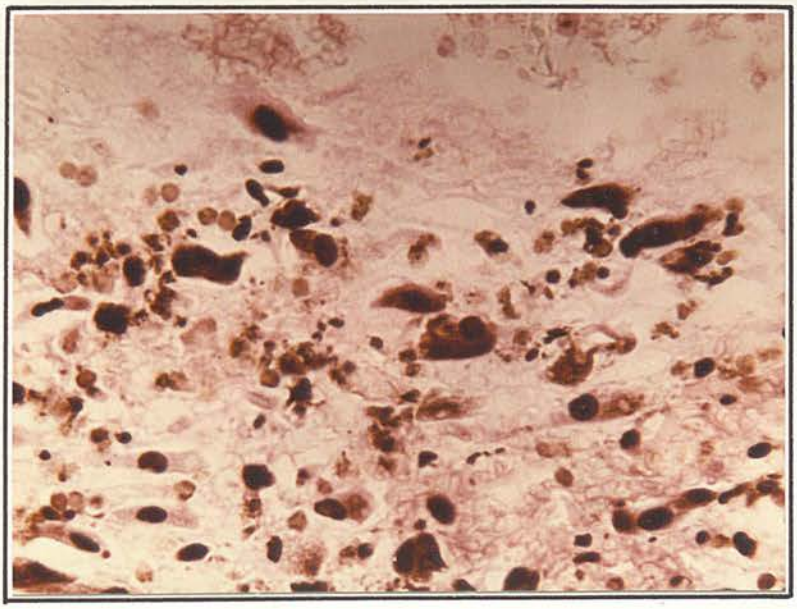


Fig. 114 X 350

Photo 115.

Marked cytoplasmic basophilia in the proliferating trophoblastic cells at the borders of some villi. Eosin Methylene Blue.

Photo 116.

Birefringent fat in the decidua in vesicular mole. Case No. 1634/57.

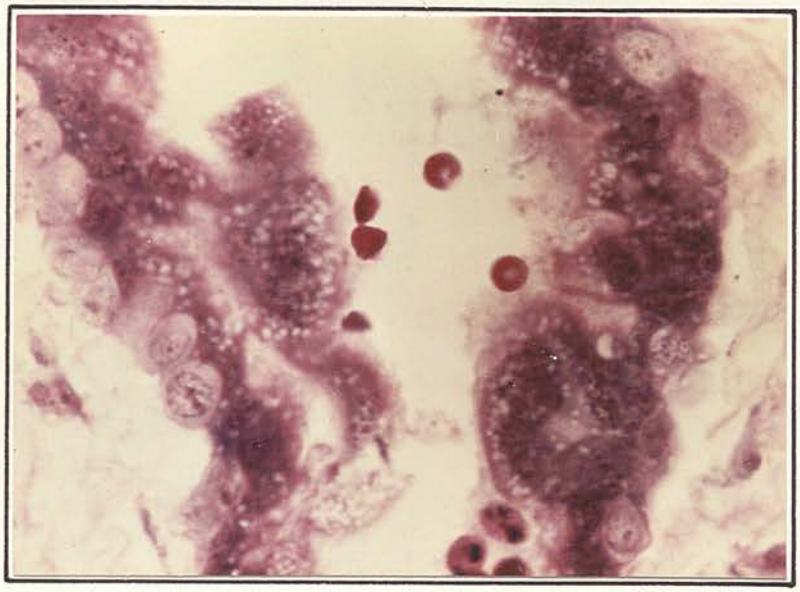


Fig. 115 X 745

Fig. 116 X 90